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MECHANISM OF CORTICOSTEROID ACTION IN DISEASE PROCESSES

BY

Oscar Hechter and Gregory Pincus (*Conference Chairmen*), S. M. Aronson, D. H. Baeder, B. L. Baker, W. R. Barclay, J. R. Bergen, M. M. Black, A. J. Butt, E. Cardona-Lynch, A. M. Dannenberg, Jr., A. Dorfman, T. F. Dougherty, R. H. Ebert, W. E. Ehrich, M. Finland, S. C. Freed, M. Friedman, G. P. Fulton, J. Gross, E. A. Hauser, H. Hoagland, C. Hunt, E. H. Kass, M. I. Kendrick, C. LeMaistre, L. M. Leonard, M. M. Lundgren, M. B. Lurie, W. McDermott, R. C. Mellinger, J. W. Rebuck, H. J. Robinson, R. H. Rosenman, D. D. Rutstein, S. S. Schneierson, J. Seifter, E. Shorr, M. H. Shulman, G. Shwartzman, A. Slocombe, A. L. Smith, H. C. Stoerk, M. Paubenhaus, L. Thomas, R. Tompsett, L. C. Wyman, P. Zappasodi, and B. W. Zweifach

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205

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MECHANISM OF CORTICOSTEROID ACTION IN DISEASE PROCESSES*

Consulting Editor: OSCAR HECHTER

CONTENTS

Introduction to Mechanism of Corticosteroid Action in Disease Processes. *By* GREGORY PINCUS and OSCAR HECHTER 625

The Influence of the Adrenal Cortex on Behavior of Terminal Vascular Bed. *By* B. W. ZWEIFACH, EPHRAIM SHORR, and M. M. BLACK 626

The Effect of Cortisone on the Vascular Reactions to Serum Sickness and Tuberculosis. *By* WILLIAM R. BARCLAY and ROBERT H. EBERT 634

The Relationship of Potassium in the Regulation of Blood Pressure with Special Attention to Corticosteroid Hypertension. *By* S. C. FREED, R. H. ROSENMAN, and MEYER FRIEDMAN 637

Direct Observations on the Circulation in the Hamster Cheek Pouch in Adrenal Insufficiency and Experimental Hypercorticalism. *By* LELAND C. WYMAN, GEORGE P. FULTON, and MAURICE H. SHULMAN 643

Head Blood Flow, Oxygen Consumption, and Electrical Activity in Relation to the Adrenal Cortex. *By* HUDSON HOAGLAND, JOHN R. BERGEN, ALAN SLOCOMBE, and CHARLOTTE HUNT 659

The Influence of Cortisone upon Granulation Tissue and its Synergism and Antagonism to other Hormones. *By* MATTHEW TAUBENHAUS 666

Evaluation of Structural and Chemical Changes in Connective Tissue. *By* JEROME GROSS 673

Mast Cells of the Omentum in Relation to States of Adrenocortical Deficiency and Excess. *By* BURTON L. BAKER 684

Evidence for the Direct Effect of Steroids on the Ground Substance. *By* JOSEPH SEIFTER, WILLIAM E. EHRICH, DAVID H. BAEDER, ARTHUR J. BUTT, and ERNST A. HAUSER 693

The Effects of Adrenal Hormones on Connective Tissue. *By* ALBERT DORFMAN 698

Interaction of Steroid Compounds and Single Cells. I. Morphological Effects. *By* LAWRENCE M. LEONARD and DAVID D. RUTSTEIN 704

Interruption by Topical Cortisone of Leukocytic Cycles in Acute Inflammation in Man. *By* JOHN W. REBUCK and RAYMOND C. MELLINGER 715

Inhibition of the Phenomenon of Local Tissue Reactivity by Corticosteroids, Salicylates, and Compounds Related to Salicylates. *By* GREGORY SHWARTZMAN and S. STANLEY SCHNEIERSON 733

Effect of Certain Corticosteroids and of Growth Hormone on Nucleoproteins of Lymph Nodes. *By* EDWARD H. KASS, MARY I. KENDRICK, and MAXWELL FINLAND 737

Cortisone and Immunity to Homoiogenous Tissue—Loss of "Individuality Differentials" from Tissues of Cortisone-Treated Rats. *By* HERBERT C. STOERK 742

Some Observations on Mechanisms of Corticosteroid Action on Inflammation and Immunologic Processes. *By* THOMAS F. DOUGHERTY 748

The Effect of Adrenal Cortical Hormones on Experimental Infection. *By* HARRY J. ROBINSON and AMELIA L. SMITH 757

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* This series of papers is the result of a Conference on Mechanism of Corticosteroid Action in Disease Processes held by the Section of Biology of The New York Academy of Sciences, October 3 and 4, 1952.

Observations on the Effect of Corticosteroids and Growth Hormone on Resistance to Experimental Pneumococcal and Influenza Virus Infections. *By* Edward H. Kass, Marguerite M. Lundgren, and Maxwell Finland 765
The Effects of Corticosteroids upon Tuberculosis and Pseudotuberculosis. *By* Charles LeMaistre, Ralph Tompsett, and Walsh McDermott 772
The Effect of Cortisone and ACTH on the Pathogenesis of Tuberculosis. *By* Max B. Lurie, Peter Zappasodi, Arthur M. Dannenberg, Jr., and Eugenia Cardona-Lynch 779
Poliomyelitis Infection by Parenteral Routes Made Possible by Cortisone. *By* Gregory Shwartzman and Stanley M. Aronson 793
Cortisone and Infection. *By* Lewis Thomas 799

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INTRODUCTION TO MECHANISM OF CORTICOSTEROID ACTION IN DISEASE PROCESSES

By Gregory Pincus and Oscar Hechter
The Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

The title of this monograph states succintly its objective. To assist us in arriving at some understanding of the basic mechanisms underlying corticosteroid action in disease processes, we are fortunate in having as contributors a group of men intensively engaged in the biochemical and physiological investigations germane to these mechanisms. Essentially, this conference is a search for information about the mode of action of substances having powerful biological effects in both normal and pathological organisms. In conducting this search, we are not looking for final answers. We certainly hope to be able to define the problem and to bring into clear focus those phenomena that most intimately represent or are intimately allied to the fundamental processes affected by the characteristic adrenocortical hormones. Our hope for result is therefore somewhat more modest than our stated objective, but "surely a man's reach should exceed his grasp." In any event, our colleagues will detail to you how the modern-day resources available to them have been employed.

These resources have been applied to problems that, a few years ago, were academic to endocrinologists and with difficulty investigated. The diverse assemblage of pathological conditions affected particularly by ACTH and the 11-oxygenated corticosteroids have created an interest that takes the problem of mechanism of action out of the academic field. Furthermore, the utility of these substances in medical practise has made them available in quantities and in degrees of purity essential to definitive investigation. In this conference, we hope to have the first harvest of the newer usages in medicine.

Following the original clinical trials with these substances, many attempts were made to correlate the known biological activities to the therapeutic effect. The result was a large number of clinical studies of the protein catabolic, electrolyte-regulating, diabetogenic, eosinopenic, lymphocytopenic, and other metabolic effects of ACTH and cortisone in diverse disease states. It has become increasingly evident from these studies that the primary therapeutic mechanisms are not directly dependent upon the general metabolic effects of the corticosteroids. Certain more specific effects are involved. It is these more direct effects that will be dealt with in this monograph.

One objective implicit in this conference, as in all symposia concerned with experimental biology, is the encouragement of discussion and critical inquiry. Problems connected with the mechanism of hormone action particularly invite such discussion and criticism. In spite of the long and honorable history of the hormones, our knowledge of their specific roles in the host of bodily processes they affect is still extremely scant. In spite of much effort, we are still faced with much bafflement. It takes men of courage and vigor, like the contributors to this monograph, to tackle such baffling problems.

THE INFLUENCE OF THE ADRENAL CORTEX ON BEHAVIOR OF TERMINAL VASCULAR BED

By B. W. Zweifach,* Ephraim Shorr, and M. M. Black†
Department of Medicine, The New York Hospital, and Department of Pathology, New York Medical College, New York, N. Y.

Many investigators have shown that a central feature of adrenal insufficiency is the profound collapse of the circulation, which develops progressively. Swingle[1] was greatly impressed with the increased capacity of the peripheral vascular tree in such cases and attributed it to a deficiency of adrenocortical secretions essential for the maintenance of "vascular tone." Used in its broadest sense, the term encompasses many facets of vascular activity and is in no way specific. Unfortunately, the experimental data thus far available do not permit a more precise evaluation of the particular homeostatic pathways through which the adrenal cortex influences the behavior of the peripheral blood vessels.

The mechanism of action of adrenal corticosteroids on the vascular system should be considered with three possible categories in view. First, does the gland produce hormones that act as blood-borne principles with a direct effect on the vascular system? Secondly, are the vascular effects referable to the basic function of the adrenal corticosteroids in the electrolyte metabolism of the smooth muscle cell? Thirdly, does the adrenal cortex influence vascular behavior indirectly through effects on other organ systems, such as the kidney?

One of the most fruitful approaches to this problem is that of direct observation of the functional behavior of the capillary bed in tissues such as the mesentery under various experimental conditions.[2, 3] This approach has made it possible to relate the observed phenomena to specific structural components of the terminal vascular bed. With this as a frame of reference, one encounters less difficulty in orienting the complex behavior of the capillary circulation in its proper perspective with reference to the regulatory influence of the adrenal cortex.

Structure and Function of Capillary Bed

The characteristic structural pattern around which the capillary bed is organized is shown in FIGURE 1. Included in the capillary bed proper are the metarterioles, the precapillary sphincters, the true capillaries, and the collecting venules. The metarterioles and precapillaries are the only structural components possessing contractile muscle elements and hence are a major factor in regulating the volume and extent of blood flow through the network of capillary vessels.[4] The true capillaries are simple endothelial tubes surrounded by a thin connective-tissue coat. As such, they are nonmuscular and show changes in caliber only secondarily to alterations in blood flow through the vascular bed.

Representative criteria of activity were selected as a measure of the functional capacity of the capillary bed,[5] as follows: (1) *Vasomotion*, a spontaneous contraction and relaxation of the metarterioles and precapillaries, is a prominent feature of the normal circulation; (2) The capacity of the metarterioles and precapillaries to *react to epinephrine*, topically applied, serves as a relative measure of the contractile capacity of the vascular smooth muscle to respond to physiological stimuli; (3) The *arterioles* normally exhibit a narrow *tonic* state. Variations in the caliber of these vessels provide an index of the activity of their vasoconstrictor–vasodilator mechanisms; (4) The true *capillaries* exhibit a state of *tone* and elasticity under normal conditions that permits them to maintain a fixed caliber in the face of wide excursions in blood flow through the bed; (5) The *venules* are also kept in a *tonic* narrowed state by virtue of partial contraction of irregularly spaced smooth muscle cells. The above phenomena show a characteristic pattern in the normal state, with deviations from this pattern of activity serving as a means of evaluating the functional

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* Present address: Department of Biology, New York University, New York, N. Y.
† With the technical assistance of Miss Delilah B. Metz.

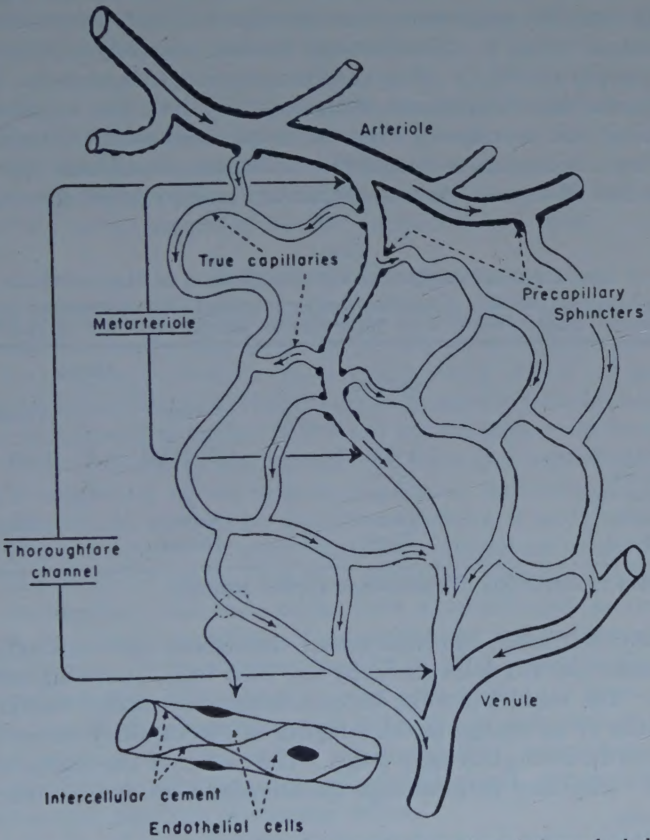

FIGURE 1. Diagrammatic representation of basic structural pattern of terminal vascular bed, as visualized in mesentery. Capillary bed includes metarterioles, precapillary sphincters, true capillaries and collecting venules. Muscular coat indicated by heavy scalloping of vessel walls.

state of the vascular bed in a variety of acute or chronic experimental syndromes.

Effects of Adrenalectomy

When the adrenals of rats are surgically removed and no supportive therapy is given, the terminal vascular bed in the mesoappendix undergoes a progressive loss in over-all functional activity. This is manifest initially by a hyporeactive state of the muscular components that develops within 24 hours after surgery, as indicated in TABLE 1. These vessels become relatively unresponsive, not only to epinephrine but to other vasoconstrictor agents as well. Within 48 hours, the muscular components of the capillary bed, the metarterioles and precapillaries, fail to respond even to direct mechanical stimulation with microneedles. Spontaneous vasomotion becomes slowed and less apparent during the first 24 hours. As a consequence, capillary blood flow is no longer preferentially restricted, but widespread throughout the capillary network. After 72 hours, the arterioles gradually lose their tonic narrowing and undergo dilatation. The venules likewise become moderately dilated within 48 to 72 hours. Little or no change in the integrity of the capillary endothelium can be demonstrated during this early stage. The caliber of the capillaries remains unchanged. Capillary permeability, as determined by dye studies, is within normal limits.

TABLE 1

EFFECT OF ADENALECTOMY ON PERIPHERAL CIRCULATION IN MESOAPPENDIX OF RAT

(Changes in functional capacity of capillary bed in untreated adrenalectomized rats. During terminal stage, animal is in progressively developing state of shock.)

	Controls	Adrenx.	
		1–3 days	Terminal Stage
Reactivity to epin.	1:4–1:6M*	1:1–1:2M	1:50–1:250M
Vasomotion	++	+	0
Arteriolar tone	++	±	0
Capillaries	Narrow	Narrow	Distended
Venules	Tonic	Dilated	Petechiae

* 1:xMillion concentration of epinephrine applied topically.

During the terminal stages of adrenal insufficiency, concomitant with the appearance of a shocklike state, many of these phenomena become accentuated. At this stage, it is difficult to distinguish between the effects of shock *per se* and adrenal insufficiency. The skin appears pale and ischemic. The arterioles in the ear, for example, are so thin and narrow as to be difficult to visualize. The visceral circulation, on the other hand, appears plethoric and congested. Vascular reactivity, which initially was depressed, now becomes significantly heightened. It is interesting that this change in vascular responsiveness develops during the period in which intra- and extracellular levels of sodium and potassium first become significantly affected. The heightened reactivity may therefore be a reflection of a loss of potassium from the smooth muscle cell. During this stage, the muscular vessels show an inability to maintain a contracted state following stimulation with epinephrine. The contraction is of short duration and is followed by a considerable dilatation. Repeated applications of epinephrine soon exhaust the capacity of the metarterioles and precapillaries to respond and they become refractory to such stimuli. Spontaneous vasomotion is no longer seen. The terminal arterioles are dilated and the capillary bed has a sluggish, over-all blood flow.

The walls of the true capillaries show an interesting sequence of changes. The endothelial cells appear to have lost their state of tone, being readily deformed by microneedles and unusually fragile. Capillary permeability becomes altered, as manifest by an increased outward diffusion of impermeable dyes, such as T-1824. The small venules are dilated and show a tendency to rupture, with numerous petechial hemorrhages being observed on the venous side of the circulation. Thus, the capillary bed now acts as an inert set of vessels without active control of the circulation through them.

Replacement Therapy

The administration of various adrenal corticosteroids to adrenalectomized rats restores the vascular bed in the mesentery to its original control state. Maintenance of adrenalectomized rats on a high intake of NaCl (1 per cent salt in drinking water) serves to prevent the derangements in vascular function that were noted in untreated animals. The NaCl-treated animals can be maintained in good health and show a normal vascular bed for at least several months. The reactivity to constrictor agents, such as epinephrine, remains within the normal range in such animals. Arteriolar tone is maintained and vasomotion, although slowed, is clearly evident. It is only when such animals are subjected to stress that the deficiency of adrenal cortical secretions becomes evident. Thus, although the capillary wall and venules have a normal appearance under the microscope, the simple expedient of increasing the temperature of the drip solution that bathes the mesentery from 37.5 to 39°C. results in the formation of numerous petechial hemorrhages. This may indicate a weakness or deficiency in one of the supporting connective-tissue structures in the vessel wall. The terminal vascular bed in salt-maintained, adrenalectomized rats deteriorates rapidly following hemorrhage and shows a complete inability to compensate for this type of vascular stress.

Administration of water-soluble cortical extract (Upjohn's 0.5–1.0 cc. per day), cortisone (1–2 mg. per day), or DCA (1 mg. per day) restores the capillary bed of adrenalectomized rats to a state indistinguishable from that of control animals. As was the case with NaCl-supported rats, animals treated with cortisone or whole extract cannot withstand stress, such as hemorrhage or cold. A transient compensatory state develops in the terminal vascular bed following hemorrhage, but this persists for only 15 to 20 minutes before vascular deterioration sets in.

Stimulation of the adrenal cortex by ACTH (200–600 γ) in normal rats is accompanied within two to three hours by increased vascular reactivity to epinephrine (1:4M to 1:100M), augmentation of vasomotion, and a definite narrowing of the terminal arterioles and metarterioles. The capillary circulation becomes restricted to preferential channels and is extremely rapid. A similar sequence of changes can be produced in the mesoappendix by the administration of comparatively large doses of cortisone for several days (3–5 mg.). Injections of DCA (either acute or chronic) into normal animals have no demonstrable effect on the reactivity of the terminal vascular bed.

Relation of Adrenals to Kidney. Many of the above phenomena can be referred to a direct vascular action of adrenal corticosteroids. There is evidence, however, to indicate that the adrenal cortex is related to the production of vasoactive materials by the kidney.[6] For example, despite adequate maintenance on salt, the kidneys of adrenalectomized animals lose the capacity to produce the vasoexcitor principle, VEM. This principle has been shown to increase the reactivity of the metarterioles and precapillaries of the mesoappendix to topical epinephrine and to augment spontaneous vasomotion.[7] The administration of DCA, cortisone, or adrenocortical extract brings about reconstitution of VEM metabolic mechanisms in the kidney. As will be shown later, this may be significant with respect to the role of the kidney and renal vasoactive substances in the genesis of hypertension.

TABLE 2

STRUCTURES AND MECHANISMS IN PERIPHERAL VASCULAR BED INFLUENCED BY ADRENAL CORTEX

(Possible modes and sites of action of adrenal corticosteroids on various components of Capillary bed.)

Arterioles	Vasomotor tone
Precapillary sphincters	Reactivity to epinephrine Spontaneous vasomotion
Capillaries	Permeability: (Endothelial cell tone, pericapillary sheath)
Venules	Perivascular coat

The effectiveness of salt administration in restoring the responsiveness of the small blood vessels in adrenalectomized animals to epinephrine and to other constrictor influences offers a highly significant clue to the mechanism of action of the adrenals in vascular homeostasis. It would appear that the maintenance of electrolyte balance between the smooth muscle cell and its environment represents a critical factor in the regulation of vascular reactivity. The above experiments emphasize that an important facet of the contribution of the adrenal cortex to the physiology of the capillaries is its function in maintaining certain renal metabolic processes in an intact state.

Experimental Hypertension

The maintenance and the development of experimental hypertension appear to be dependent upon the presence of the adrenals.[8] Renal hypertension cannot be produced during adrenal insufficiency in either the rat or dog. In these instances, an increased vascular reactivity does not develop in the mesenteric circulation, as it does in normal animals subjected to identical experimental manipulation of the kidneys. Adrenalectomized rats maintained on NaCl likewise do not develop a sustained elevation in blood pressure following renal manipulation. As mentioned previously, the kidneys of these animals cannot produce the vasoexcitor principle, VEM. Supportive therapy with DCA or cortisone in such animals results in the rapid development of a typical hypertension. Vascular reactivity likewise becomes elevated, concomitant with the rise in blood pressure. It is difficult to ascertain the precise extent of the adrenal contribution to experimental hypertension; whether it has a direct effect on vascular activity, or whether its action is through an influence on renal metabolism.

Hypertension can be produced experimentally in rats by the administration of large doses of DCA and salt. This type of hypertension is not accompanied by the typical vascular changes that were observed in animals in experimental renal hypertension.[9] It should be noted, however, that adrenalectomized dogs maintained in good health by two DCA pellets (75 mg. each) will develop and sustain a typical hypertension following renal manipulation. Thus, DCA will maintain renal humoral mechanisms in an intact state, upon which the hypertensive effects of renal manipulation can then be superimposed. Excessive doses of DCA *per se* however do not either stimulate the over-production of renal vasoactive principles or influence the reactivity of the terminal vascular bed.

Tetrazolium Studies. Although the above type of evidence is suggestive, it cannot be offered as evidence for the active participation of the adrenal gland in the development of the hypertensive syndrome. None of the conventional indices of adrenocortical function, such as determinations of corticosteroids in urine and in blood, or the typical eosinophil response, have brought to light evidence of adrenal hyperfunction. The histologic evidence is likewise equivocal. We have employed a somewhat different approach as a means of evaluating adrenocortical secretory activity. Recent experiments have shown that it was possible to assess the functional state of the adrenal cortex by a new histochemical technique involving the metabolic interaction between enzyme systems of the living cell and an organic dye, 2,3,5-triphenyltetrazolium chloride (TTC).[10] TTC is a colorless, water-soluble compound whose oxidation–reduction potential is such as to permit it to act as a hydrogen acceptor and, thereby, to be reduced to an insoluble red form by intracellular dehydrogenase enzymes. In the case of the adrenal cortex, the red color is taken up by the intracellular lipid granules. Adrenal slices from normal animals, incubated with TTC, show a uniform and characteristic deposition of red color throughout the three zones of the cortex. In conditions of decreased adrenal function, TTC reductase activity is either reduced or completely absent. In conditions of hyperfunction, the deposition of TTC becomes accentuated. The reduction of TTC by different zones of the adrenal can be suppressed by specific metabolic inhibitors, such as malonate or fluoride.

This type of histochemical approach was employed in studies of the adrenals of normotensive and hypertensive animals.[11, 12] The intact adrenals incubated in TTC showed a uniform staining, irrespective of whether the animal was normotensive or hypertensive. A striking difference between the two sets of animals was brought to light, however, when the adrenals were incubated in the presence of either malonate or fluoride (M/4). The outermost zone, the glomerulosa, of the hypertensive was found to be resistant to the inhibitory action of both malonate and fluoride, and continued to reduce TTC, as shown in FIGURE 2. In many instances, the reduction of TTC actually appeared to be enhanced rather than to be depressed. This was in definite contrast to the adrenals of normal animals, in which the reduction of TTC by the glomerulosa was completely abolished. This difference between normal and hypertensive adrenals represents a qualitative metabolic disturbance, since incubation of hypertensive adrenals in the presence of as much as ten times the original concentration of inhibitor had no depressant effect upon TTC reductase activity in the glomerulosa.

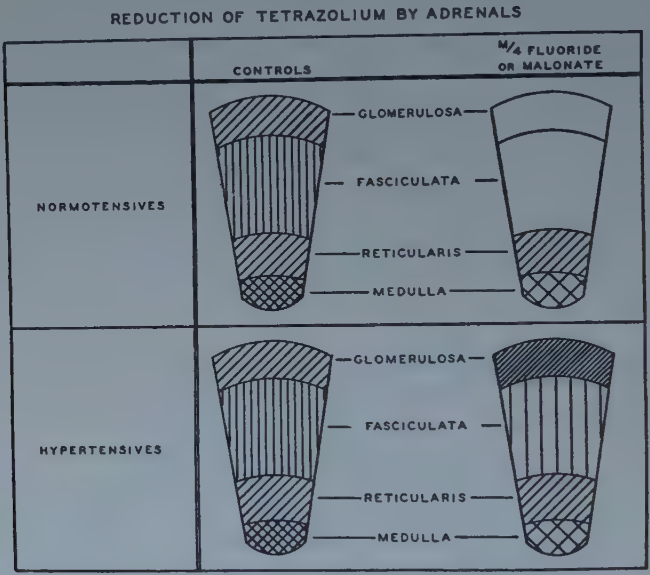

FIGURE 2. Alterations in capacity of adrenal tissue to reduce TTC on incubation *in vitro*. Intensity of staining is indicated by cross shading. Heavier shading signifies augmented deposition of TTC. Wider spacing of cross-hatching indicates diminished coloration of zone by TTC. No shading indicates complete suppression of TTC activity. Note difference between controls and hypertensives in inhibitory effect on glomerulosa by metabolic inhibitors, fluoride, and malonate.

Here, again, the evidence of adrenocortical involvement does not establish the extent to which metabolic alterations in the glomerulosa contribute to the development of hypertension. Also, whether the alteration in the adrenals represents a primary change which, in turn, affects the production of vasoactive substances by the kidney, or whether a change in renal metabolism secondarily affects the adrenals cannot be answered in the light of present evidence.

Summary

In TABLE 2 are listed the possible sites and modes of action of the adrenal cortex with respect to the capillary bed.

(1) Arteriolar tone is definitely affected by adrenal ablation.

(2) Both spontaneous vasomotion and the responsiveness to epinephrine of the precapillary sphincters are markedly influenced by the adrenal cortex.

(3) The capillaries themselves are affected late in the syndrome, with changes being manifest in the tone of the endothelial cells and in the fragility of the supporting pericapillary connective-tissue layer.

(4) The muscular venules show a pronounced tendency toward rupture, presumably because of a deficiency in the perivascular connective-tissue coat.

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THE EFFECT OF CORTISONE ON THE VASCULAR REACTIONS TO SERUM SICKNESS AND TUBERCULOSIS

By William R. Barclay and Robert H. Ebert
The University of Chicago, Chicago, Ill.

The rabbit-ear chamber provides a convenient method for studying small blood vessels at high magnifications and for long periods of time. This technique had been employed to study the course of tuberculous infection,[1] the focal reaction, and serum sickness.[2] This technique has also revealed, at least in part, the manner in which cortisone modifies the inflammatory response of tissues to infection[3] and to hypersensitivity states.[4, 5] The technique and the tissue changes described here were illustrated by a Kodachrome motion picture

The first portion of the film illustrates the mechanical construction of the chamber and the method of making observations. The chamber itself is made of lucite[6] and consists of a base plate with a raised central observation table, a mica cover slip, and a lucite cover-slip-retaining ring. The chamber is inserted into the pinna of the rabbit with a strictly aseptic technique. At the time the chamber is inserted, a clot is formed on the central observation table and, in the ensuing three to four weeks, blood vessels grow into this clot and transform it into a thin vascularized layer of connective tissue.

The normal vascular pattern in such a chamber is composed of arterioles, venules, and capillaries supported by an undifferentiated layer of connective tissue. The arterioles have a well-developed muscular wall, a highly refractile endothelium, and a narrow uniform bore. The muscular wall is actively contractile, and arterioles periodically show complete constriction for short periods of time. The venules are wide channels, not of uniform diameter, and tend to be somewhat tortuous. The capillaries have only a thin endothelial wall, a narrow diameter, and are intermittently closed off by precapillary sphincters.

Effect of Cortisone on Serum Sickness

Two ear-chamber rabbits, weighing 4500 grams, were treated as shown in FIGURE 1.

Normal horse serum (10 ml. per kilogram of body weight) was injected intravenously on day 0, day 18, and day 39. Cortisone (5 mg. i.m. once daily) was given for the first 22 days, then discontinued, and restarted on day 46 after the third injection of horse serum.

The first injection of horse serum produced a slight increase in the vascularity of the chambers. The venules became slightly dilated, but the arterioles retained their normal tone and general appearance. Slight sludging of erythrocytes occurred, and a few leucocytes were seen sticking to the endothelium of the venules. By the fifth day, these minimal changes had disappeared and the vascular pattern appeared normal.

The second injection of horse serum on day 18 caused moderate sludging of erythrocytes, slight dilatation of venules, and slight-to-moderate sticking of leucocytes to the endothelium. There was no alteration in arteriole tone, however, and no leucocytes were observed sticking to arteriole endothelium.

Following the cessation of cortisone administration on day 22, the typical changes of serum sickness as observed in the ear chamber[2] were seen. The most notable change was in the tone of the arterioles, which became dilated three to four times and showed moderate stickiness of the endothelium toward the leucocytes. The venules also became more dilated, and the general vascularity of the chamber increased.

The third injection of horse serum on day 39 to animals not receiving cortisone produced marked arteriole, venule, and capillary dilation, marked sludging of erythrocytes, and marked sticking of leucocytes to arteriolar endothelium. The reinstitution of cortisone treatment on day 46 completely reversed the arteriole changes and partially reversed the damage to venules and capillaries.

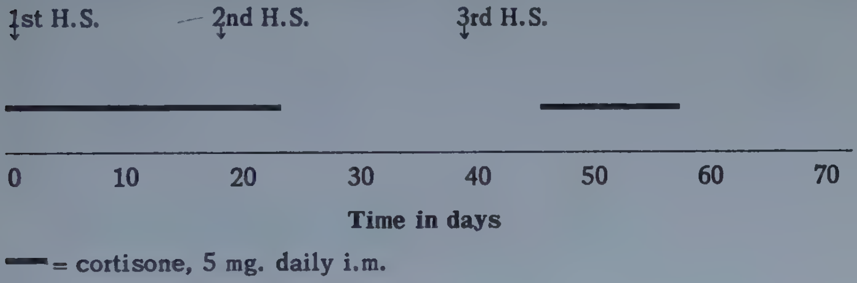

FIGURE 1

It would appear therefore that cortisone is capable of suppressing the vascular damage that occurs in serum sickness in rabbits and, in particular, the damage to arterioles. Cortisone is also capable of partially reversing the changes that have been established in serum sickness.

Effect of Cortisone in Tuberculous Infection

In the ear chamber, an active tuberculous infection is characterized by a necrotic avascular area surrounded by a zone of inflammation. The arterioles are dilated, the venules are widened and tortuous and a great many capillary channels are open. Marked hemoconcentration, sticking of leucocytes to venous endothelium, and free fluid and white cells in the intervascular spaces are seen. Tuberculous infection is also characterized by venous thromboses in vessels immediately adjacent to the extending necrotic area.

Cortisone (5 mg. i.m. daily) restores the vascular pattern toward normal. The arterioles regain their normal tone, the venules show moderate dilatation, and many of the capillary channels close off so that the appearance of hypervascularity associated with inflammation disappears. Thrombosis of small venules still occurs, however, and sticking of leucocytes to the venous endothelium is still in evidence. Moreover, although the surrounding vascular tissue appears relatively normal, the area of caseation extends during the treatment with cortisone. When cortisone is discontinued, the inflammatory reaction about the area of caseation flares up, and venous thromboses, extravascular hemorrhages, and exudation of fluid and cells occur. In fact, instituting cortisone treatment and then withdrawing it seems to accelerate the destruction of tissue by accelerating the extension of the caseation necrosis.

Summary

The effects of cortisone seem to be nonspecific with regard to the type of inflammatory changes studied. In the normal chamber, cortisone increases the general vascular tone. In serum sickness and in tuberculous infection, cortisone suppresses vascular dilatation, particularly of arterioles, and lessens damage to vascular endothelium, as measured by sticking of leucocytes and extravasation of fluid and cells. The changes produced by cortisone are reversible and the inflammatory reaction recurs when cortisone is discontinued.

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THE RELATIONSHIP OF POTASSIUM IN THE REGULATION OF BLOOD PRESSURE WITH SPECIAL ATTENTION TO CORTICOSTEROID HYPERTENSION*

By S. C. Freed, R. H. Rosenman, and Meyer Friedman
Mount Zion Hospital, Harold Brunn Institute, San Francisco, California

In recent years, the potassium cation has received considerable attention in regard to many phases of cellular metabolism. Among the widespread effects noted are those on the circulatory system. It is recognized clinically that potassium deficiency may induce increased heart size, abnormal cardiac function, and electrocardiographic changes. The effects of this deficiency upon the peripheral vasculature and upon the blood pressure have failed to receive adequate study, however, despite the apparently frequent occurrence of hypotension in clinical states associated with lowered potassium levels. The latter problem has been investigated in our laboratory during the past few years.

Initially, it was found that deficient potassium levels in the rat were associated with a significant fall in blood pressure.[1] Thus, when potassium was rigidly restricted from the diet of intact rats, their blood pressures fell appreciably after the third week. At that time, the average systolic blood pressure of 29 such rats was 90 mm. Hg, compared to an average of 111 mm. Hg in 29 control animals. This fell progressively, reaching an average of 84 mm. Hg at the seventh or eighth week (FIGURE 1). This hypotensive response often was associated with a low and irregular cardiac rhythm, as detected by the microphonic manometer. Moreover, this hypotensive effect could not be ascribed to subnormal weight gain. Other rats with a comparable poor weight gain due to the feeding of a restricted amount of stock diet failed to exhibit a depressor response. Similar changes were not observed in rats fed a sodium-deficient diet. Indeed, simultaneous restriction of both sodium and potassium appeared to prevent the depressor response occurring in rats fed a diet deficient only in potassium.[2]

The effect of dietary potassium deprivation upon the blood pressure was also studied in rats made persistently hypertensive by the Grollman "figure-of-eight" technic of renal compression. As in the intact rat, a depressor response was observed (Figure 2). Within seven weeks after ingestion of the potassium-deficient diet, the blood pressures of 37 such hypertensive rats fell from an average of 162 mm. Hg to an average of 122 mm. Hg. Seventeen control hypertensive rats receiving the same diet supplemented with 0.5 per cent potassium chloride showed a gradual further rise of blood pressure from an initial average of 155 mm. Hg to 175 mm. Hg at the seventh week.[3]

Experiments then were designed to test the specificity of the potassium ion in this depressor response.[4] Eighteen intact rats were fed the potassium-deficient ration for 12 weeks, at which time their average blood pressure had decreased to 86 mm. Hg. Their serum potassium concentrations averaged 2.9

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* Aided by grants from the American Heart Association, the San Francisco Heart Association, the United States Public Health Service, and the Life Insurance Medical Research Fund.

meq., compared to 5.59 meq. in 11 control rats. A similarly treated group of 13 rats with an average blood pressure of 82 mm. Hg were injected subcutaneously with 10 ml. of isotonic KCl and were then permitted to drink one per cent KCl solution *ad libitum* for 24 hours, during which all food was withheld. Their blood pressures rose to an average of 111 mm. Hg during this interval, and their average serum potassium concentrations rose to 6.4 meq./l. Since the administration of potassium was associated with a rapid and significant rise of blood pressure without an improved nutritional status, it was felt that the induced potassium deficiency was the specific factor leading to the fall in blood pressure. In somewhat similar fashion, a group of five hypertensive rats were fed the potassium-deficient diet. Their average systolic pressure fell from 159 mm. Hg to 136 mm. Hg at the eighth week. When potassium was added to the diet of the experimental group, their average pressure rose rapidly, reaching 174 mm. Hg within 2 weeks.

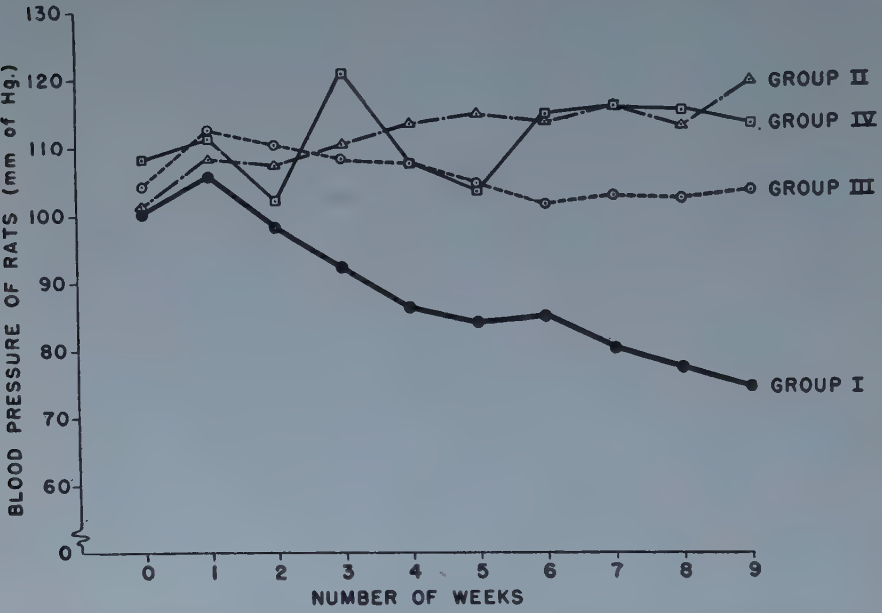

FIGURE 1. The effect of a K-deficient diet (0.006% K^+) on average blood pressure of rats. Group I, K-deficient synthetic diet; Group II, stock diet; Group III, restricted stock diet; Group IV, synthetic diet with added potassium (0.5% KCl).

In the final analysis, the causal mechanism of this depressor response might be (1) a decreased cardiac output; (2) a lowered peripheral vascular resistance; or (3) a combination of both factors. It seems likely that cardiac output is diminished. The heart beat of the potassium-deficient rat usually is slow and less vigorous than normal. Myocardial weakness is associated with a low myocardial potassium content. Cardiac contractility of the isolated heart is decreased in the presence of low potassium levels. On the other hand, the smooth muscle tonus of the urinary bladder and gastrointestinal tract is lessened in potassium-deficient animals. A similar loss of tone might be found in the smooth muscle of the peripheral vasculature.

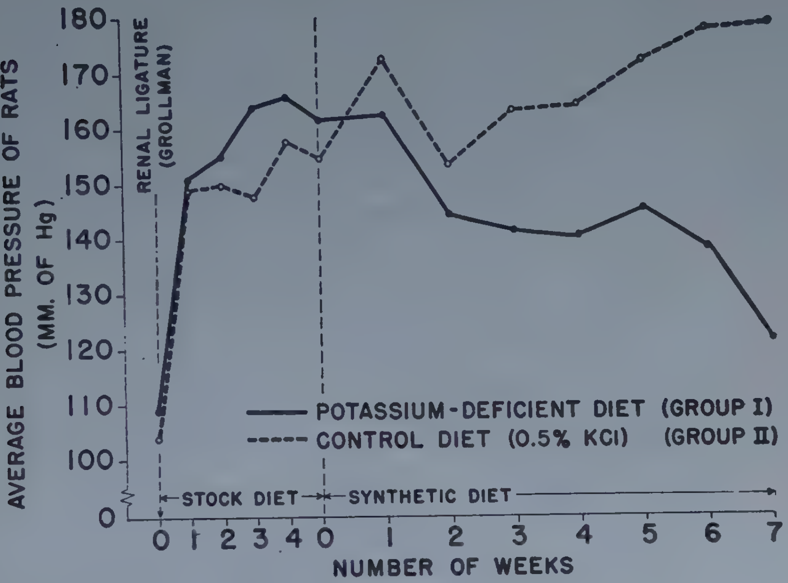

FIGURE 2. The effect of potassium-deficient diet upon blood pressure of hypertensive rats.

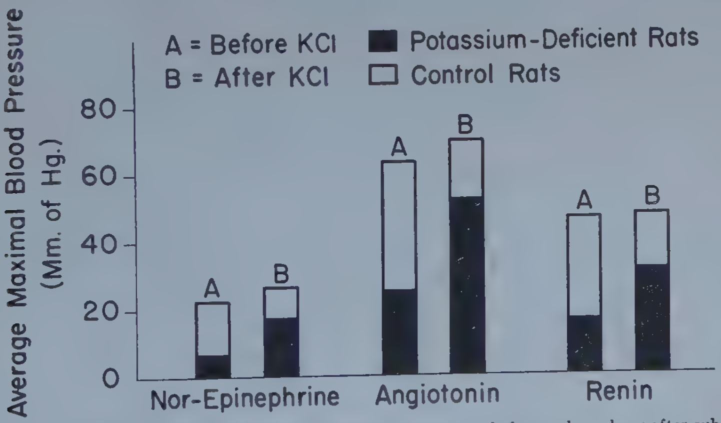

FIGURE 3. The average pressor response of potassium-deficient rats before and one hour after subcutaneous injection of KCl.

In order to test this latter possibility, the peripheral vascular reactivity of potassium-deficient rats was studied.[5] It was found that the pressor responses to intravenously injected epinephrine, nor-epinephrine, angiotonin, and renin were considerably decreased in these rats, being one-third to one-half those of the control animals. One hour after the subcutaneous administration of isotonic potassium chloride, the pressor responses of the potassium-deficient rats were considerably elevated (FIGURE 3). These studies showed that this loss of peripheral vascular reactivity was rapidly reversible. Moreover, the return toward normal vascular reactivity following potassium administration was associated with an increase in blood pressures and of serum potassium concentrations to more normal levels.[6] The results of these experiments suggested that the depressor response occurring during dietary-potassium deprivation may be related at least in part to associated peripheral vascular changes.

Numerous studies have implicated the adrenal cortex in certain hypertensive states. The adrenal cortical steroid found to be most active in relation to the blood pressure is desoxycorticosterone. It is generally believed that excess sodium ion is essential for a pressor response to desoxycorticosterone administration. Our studies suggest, however, that the potassium ion may also play a significant role in this pressor response.[7]

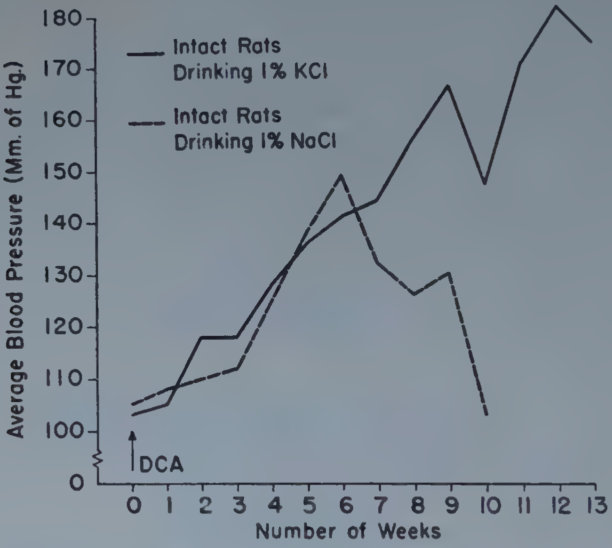

FIGURE 4. The effect of DCA on intact rats given supplemental potassium or sodium.

Two groups of intact rats were fed a stock diet and were injected with 4 mg. of DCA three times weekly. One group received one per cent NaCl solution as their drinking fluid and the other one per cent KCl solution. Significant differences in the course of their weekly blood-pressure values were observed. An initial rise of blood pressure occurred in both groups, but the rats drinking NaCl solution later sustained a fall in their blood pressures from an average of 148 mm. Hg at the sixth week to 105 mm. Hg at the tenth week (FIGURE 4). At this time, their average serum potassium concentration was well below normal at 3.4 meq./l. Those rats drinking one per cent KCl solution did not show this fall in blood pressure, which continued to rise to an average of 164 mm. Hg at the tenth week. The average serum potassium level of these animals was normal (5.6 meq./l.).

The importance of adequate potassium for the pressor response of DCA was further demonstrated in intact potassium-deficient rats. Administration of DCA to such animals failed to induce even the slightest pressor response. Indeed, in many of these rats the blood pressures fell promptly to further hypotensive levels. The addition of one per cent sodium chloride to the drinking fluid of such potassium-deficient rats receiving DCA caused even more marked depressor responses. Since the dosage of DCA often used for the induction of hypertension causes a marked excretion of potassium, it seems probable that some of the inconsistencies and even failures to induce hypertension following DCA administration might be related to the loss of this cation in quantities sufficient to negate the possible pressor action of this steroid.

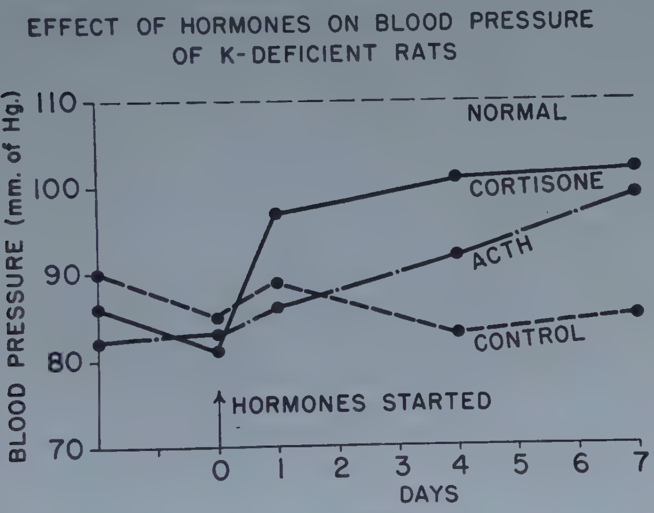

FIGURE 5. Effect of hormones on blood pressure of K-deficient rats.

The failure of DCA to restore the lowered blood pressures of potassium-deficient rats was in marked contrast to the response to cortisone. Twelve intact rats were fed a potassium-deficient diet until significant decreases of blood pressure were observed. The subcutaneous administration of 1 mg. of cortisone daily was associated with a significant rise of their average blood pressure from 82 mm. Hg to 101 mm. Hg within a three day period (FIGURE 5). The animals remained in the same nutritional state, and the average value for serum potassium (3.25 meq./l.) was about the same as in the untreated potassium-deficient control rats (3.4 meq./l.). Likewise, the administration of one mg. ACTH, twice daily, raised the average blood pressure of 12 potassium-deficient rats from 83 mm. Hg to 99 mm. Hg after seven days, without altering the potassium level of blood.

The nature of these responses has not been established as yet. The heart rates of the treated animals are faster and more regular than those of the untreated ones, indicating a possible action on improving myocardial contraction. It may also be associated with a more vigorous peripheral vascular response since others have found that cortisone apparently increases the sensitivity of the peripheral vascular system to pressor agents.

It is also of some interest that the potassium-deficient rat resembles superficially an adrenocortical-deficient animal. It is asthenic, hypotensive, and, as has been shown for the adrenalectomized animal, also has a diminished vascular response to pressor agents. Cortisone seems to correct some of these features, especially those related to the cardiovascular system. The administration of ACTH has a similar effect. This would suggest that the adrenal cortex of potassium-deficient rats remains responsive to ACTH stimulation.

Whether or not potassium-deficiency results in an actual suppression of adrenal cortex activity remains debatable. If one accepts the depression of blood pressure by nutritional means as an indication of cortical insufficiency, then one could consider that restriction of potassium is most effective in this regard. We must, however, acquire more direct evidence to establish this possible relationship and how much this might contribute to the depressor effect of potassium restriction.

Summary

(1) Rigid restriction of dietary potassium induces a fall in the blood pressures of both normal and hypertensive rats. The depressor effect is a specific response to the potassium deficiency and is promptly reversible. It is associated with a subnormal peripheral vascular response to pressor agents.

(2) An adequate supply of potassium is essential to the development of DCA hypertension. DCA is unable to normalize the reduced blood pressure of potassium-deficient rats.

(3) Cortisone and ACTH induce elevations of blood pressure toward normal values in potassium-deficient rats without altering the blood levels of potassium.

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DIRECT OBSERVATIONS ON THE CIRCULATION IN THE HAMSTER CHEEK POUCH IN ADRENAL INSUFFICIENCY AND EXPERIMENTAL HYPERCORTICALSIM[*]

By Leland C. Wyman, George P. Fulton, and Maurice H. Shulman
Department of Biology, Boston University, Boston, Massachusetts

The present investigation was undertaken to study, by direct microscopy, the relationship between the functional state of vascular smooth muscle and conditions of deficiency or excess of the adrenocortical hormones, as well as the role of corticosteroids in maintenance of the integrity of the vascular endothelium. For this purpose, techniques developed in our laboratory have been utilized (Lutz, Fulton, and Akers, 1950), especially the method of transilluminating the everted cheek pouch of the golden hamster (*Mesocricetus auratus*) for cinephotomicrographic recording of significant changes in blood flow or blood-vessel walls, during adrenal insufficiency or during experimental hypercorticalism. Cortisone acetate and desoxycorticosterone acetate were employed as examples of the two important kinds of steroids. Electrocardiograms were recorded by means of a preamplifier (Grass Instrument Company) and oscilloscope. The sensitivity of vascular smooth muscle to electrical stimulation was determined by means of a custom-built precision stimulator (Grass Instrument Company), which permitted independent variation of voltage, duration, interval between stimuli, and frequency. Silver–glass microelectrodes (Fulton, 1941), 5 to 10 microns in diameter at the tip, were applied to the walls of arterioles with an Emerson micromanipulator (PLATE 1, FIGURES A and B). Injurious electrical shocks were used to test the integrity of the endothelium, white thromboembolism formation being the criterion for detecting the threshold for damage. The micromanipulator was used also to place micropipettes, which delivered probably less than 0.05 cc., in order to apply nor-epinephrine[†] directly to the walls of arterioles. The susceptibility of the blood vessels of the cheek pouch to petechial formation was tested by the application of a suction cup, 1.4 cm. in diameter, attached to a negative pressure device (PLATE 1, FIGURE C), using a modification of the method described by Shulman and co-workers (1950). Hematology was studied by the usual methods.

Vascular Effects of Adrenalectomy

A total of 60 male hamsters (8 to 10 weeks old, averaging 80 to 100 grams) were adrenalectomized bilaterally by a dorsolateral approach. The cheek pouch was everted and transillumined under nembutal anesthesia (6–10 mg. per 100 g. body weight). The small blood vessels were observed at 24 hour intervals until death, under microscopic magnifications of 15, 100, 200, and 1200 ×. The survival time without the addition of sodium chloride to the drinking water was 5 to 7 days, as reported originally by Snyder and Wyman (1951a). Unlike the rat, the adrenalectomized hamster does not live longer on an increased salt intake. This is related to the maintenance of a relatively normal serum sodium level in the plasma of the hamster after adrenalectomy. The serum potassium level, however, remains high (over 100 per cent,* suggesting that potassium retention is a significant factor in adrenal insufficiency in this species (Snyder and Wyman, 1951b).

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[†] "Levophed," kindly supplied by Winthrop-Stearns, Inc.

Slow Blood Flow and Stasis. During the early postoperative period and even prior to impending death, no marked deviations from the normal vascular picture, except for vasoconstriction and occasional vasomotion (see below), were detected by microscopic examination. The arterioles were relatively narrow and the blood flow was rapid. The venules were free from stasis and without petechiae.

Within a period of 24 hours before death, the rate of blood flow through the cheek pouch decreased significantly (observed in 36 of 42 animals examined). At this time, the pouch was dry and sticky. The larger venules became packed with slowly moving corpuscles. Stasis predominated in the capillaries and venules in all portions of the pouch. This decrease in blood flow has been recorded cinephotomicrographically (PLATE 1, FIGURE E). Bergen, Hunt, and Hoagland (1952) have reported a decrease in blood flow through the brain of the adrenalectomized rat, measuring the venous drainage as an index of rate of flow. Our microscopic observations in the cheek pouch of the adrenalectomized hamster indicate by a direct method that slow blood flow accompanies severe adrenal insufficiency.

It may be important to note that the stasis and decrease in blood flow is most prominent immediately upon eversion of the cheek pouch and prior to manipulative procedures and the act of covering the preparation with mammalian Ringer's solution. Frequently, resolution of a considerable amount of stasis occurred within 10 minutes after eversion of the pouch. An explanation for the partial recovery may be an osmotic uptake of water from the Ringer's solution covering the relatively dry pouch. For some time, this slow-flow effect of adrenalectomy was not detected in our work, because the change is gradual until death and, possibly, because of our procedure of keeping the cheek pouch moist with Ringer's solution from the moment of eversion. Perhaps, this may account for the lack of previous direct microscopic confirmation of the reports, based on indirect evidence, of increased vascular permeability in adrenal cortical insufficiency.

The decrease in rate of blood flow is not correlated with or paralleled by the occurrence of white thromboembolism (platelet or leukocytic aggregates), leukocytic margination, aggregation of circulating erythrocytes, or petechial formation. Chambers and Zweifach (1947) reported no visible alterations in the endothelial wall of capillaries in the mesoappendix of the adrenalectomized rat until circulatory collapse preceding death. Consequently, they minimized the possibility that an increased capillary permeability might account for the hemoconcentration of adrenalectomy and proposed, as an explanation, loss of tone of the vascular smooth muscle, cessation of vasomotion, and resultant inadequate venous return. In the cheek pouch of the adrenalectomized hamster, arterial vasoconstriction becomes progressively prominent until death (see below). Possibly, the blood vessels of active surface tissues are affected differently from those of inert supporting membranes in visceral areas. This would be consistent with the often repeated opinion that severe adrenal insufficiency is a shocklike condition. Peripheral vasoconstriction is a well-known characteristic of secondary shock. The slow blood flow in the cheek pouch may be caused in part by an altered permeability of the small blood vessels, and we intend to test this concept experimentally.

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* Mean serum potassium of normal or blank-operated male hamsters, 8.5 to 8.8 meq./liter; that of adrenalectomized males 4 to 7 days after operation, 19.2 to 20.0 meq./liter.

Important factors contributing to the decreased blood flow are sustained bradycardia and irregularities in the force of the heart beat and the conduction mechanism, as shown by analysis of electrocardiograms taken periodically after adrenalectomy (PLATE 1, FIGURE F). The heart rate of the hamster anesthetized with nembutal ranges from 350 to 450 beats per minute. The normal electrocardiogram possesses the P wave and a typical QRST component like that of man. In the adrenalectomized hamster, the heart rate was decreased to as low as 150 beats per minute at terminus. The electrocardiograms were similar to those of other mammals during potassium poisoning and closely resembled those accompanying hyperpotassemia in Addison's disease.

Since the stasis and decreased rate of blood flow might be due to a general debility because of reduced food intake in the adrenalectomized hamster rather than to cortical insufficiency, experiments to control this possibility were performed. A group of 20 hamsters was placed on a simulated adrenalectomy diet decreasing progressively in amount of available food as determined by careful prior measurements of daily food intake after adrenalectomy. At daily intervals, microscopic observations were made on the condition of blood flow. No significant amount of slow blood flow or stasis was observed during the time interval comparable with the life expectancy of an adrenalectomized hamster (5–7 days). At the time of death (9–14 days), slow blood flow was observed.

Involvement of the adrenal cortex in slow flow is indicated by the reversibility of this phenomenon during replacement therapy with corticosteroids. Adrenalectomized hamsters were examined for slow flow in the cheek pouch on the 10th, 12th, and 15th postoperative days, during maintenance with 1 mg. of cortisone acetate or DCA per day. The rate of flow appeared normal during this period. Balourdas and Chambers (1952) described a decreased blood flow in the mesoappendix of rats with intact adrenals during starvation and reported a betterment during treatment with cortisone and also ACTH.

Status of Vascular Smooth Muscle. The arterioles of adrenalectomized hamsters became progressively constricted from 3 to 4 days after operation until death (observed in 18 of 35 animals examined). The vascular smooth muscle did not lose tone, as reported by Chambers and Zweifach (1947) for the mesoappendix of the rat. In the hamster cheek pouch, the constricted portions of arterioles often produced a sausagelike appearance. In several instances (6 of

51 animals examined) limited portions of arterioles exhibited spontaneous rhythmic constrictions and dilations ("rapid vasomotion"; PLATE 2, FIGURES A and B). In spite of the small number of instances of this phenomenon, we feel that it is significant because, in our experience, this type of oscillation of caliber has not been observed at all in the cheek pouch of normal hamsters under nembutal anesthesia. As mentioned above, the blood vessels of the surface tissues, including the cheek pouch, may differ in their activities and reactions from those of visceral areas, thus accounting for the differences between our observations and those of Chambers and Zweifach (1947). This is supported, moreover, by an old observation reported by one of us in 1932 (Wyman and tum Suden). In this report, well before the term "vasomotion" was coined, oscillations in caliber of the arteries on the surface of the intestines and in the mesentery of rats under urethane anesthesia were described (*ibid.*, No such oscillations were seen in the vessels of the rat's ear.

It is possible that the oscillations in caliber observed by Wyman and tum Suden (1932) in the visceral vessels of the rat and by us in the cheek-pouch vessels of the adrenalectomized hamster, which we call "rapid vasomotion," are a different phenomenon from the vasomotion described by Chambers and Zweifach (1944). This view is supported by the following differences. According to Chambers and Zweifach (1944; 1947) the dilator and constrictor phases of vasomotion in the metarterioles of the rat's mesoappendix alternate irregularly from minute to minute and are relatively slow, time-lapse motion pictures being best for recording them. In their table of the duration of the phases in a single vessel over a period of 28 minutes, the dilator phases varied from 20 to 420 seconds and the constrictor phases from 15 to 120 seconds, no two complete cycles being alike. Complete cycles varied from 35 to 540 seconds. On the other hand, Wyman and tum Suden (1932) found that the rates of oscillation were not only fairly regular, but that they were quite similar even in different animals. Moreover, they were more rapid than those reported by Chambers and Zweifach, the dilations of 75 measured cycles averaging 7.4 seconds and the constrictions averaging 5.77 seconds, the total time of a cycle being about 13 seconds. In the hamster, the duration of the cycles was similar, the dilator phase lasting about 7 seconds and the constrictor phase from 3.5 to 5 seconds. The regularity of the cycles in the hamster was likewise similar to that seen in the rat. Another difference is that Chambers and Zweifach (1947) described the vasomotion as "wavelike," while Wyman and tum Suden (1932) determined by means of simultaneous observations made by two persons through a double eyepiece that the oscillations were synchronous throughout a single artery rather than being waves of constriction. Again, the character of the movements seen in the adrenalectomized hamsters resembled that reported by Wyman and tum Suden. Until further study determines either the identity or the nonidentity of the phenomena seen by Chambers and Zweifach and by us, we prefer to call the latter "rapid vasomotion."

The sensitivity of the arteriolar smooth muscle to electrical stimulation was determined in 9 adrenalectomized hamsters by means of microelectrodes.

The threshold in the normal hamster with intact adrenals was a single shock of 3 volts and 10 milliseconds duration. In 7 of the adrenalectomized hamsters, the threshold was a single shock of 1.9 to 2 volts and 10 milliseconds duration.

Fritz and Levine (1951) reported a decreased sensitivity of vascular smooth muscle and a loss of the constrictor response to repeated topical applications of nor-epinephrine in the mesoappendix of the adrenalectomized rat. We have investigated the effect of nor-epinephrine applied by micropipette directly to the walls of arterioles in the hamster cheek pouch. The threshold range for vasoconstriction in 13 normal hamsters varied from concentrations of 1:1,500,000 to 1:2,500,000 and remained constant during repeated determinations over a period of three hours. In 12 adrenalectomized hamsters, from 4 to 7 days after operation, the range varied from 1:900,000 to 1:1,000,000 and the sensitivity decreased still further with time. The sensitivity to electrical stimulation, however, was maintained.

The pouch of the adrenalectomized hamster remained relatively pale until death, because of the predominating arteriolar constriction. A possible explanation for this vasoconstriction may be found in the prevailing hyperpotassemia. There is evidence that hyperpotassemia produced by K solutions can result in prolonged coronary constriction in dogs (Katz and Lindner, 1938) and similar findings have been reported in skeletal muscle vessels (Dawes, 1941). High plasma K has been reported in the terminal stages of shock by a number of investigators (see Overman, 1951) and this condition is accompanied by peripheral vasoconstriction. Moreover, contraction or contracture of various types of muscle from both vertebrates and invertebrates has been found to be produced by treatment with potassium (Heilbrunn, 1943). A similar explanation might be invoked to account for the vasoconstriction occurring in cortisone treated hamsters (see below) because increased plasma and erythrocyte K concentrations have been reported in cortisone treated normal dogs (Overman, 1951). We intend to investigate the blood potassium in cortisone treated hamsters.

Petechial Formation. Spontaneous petechiae were rarely found in the cheek pouch of the normal hamster. In a few instances, 1 to 5 petechiae were counted in the entire pouch at a magnification of 15 ×. Most of these were the result of previous handling of the pouch, as indicated by proximity to former pin holes. Other petechiae were undoubtedly the result of an unrecognized abnormal condition of the hamster, such as an unsuspected infection.

The susceptibility of the blood vessels of the cheek pouch to petechial formation was tested by the negative-pressure device. In a series of normal hamsters, a negative pressure of 300 mm. Hg, applied for 60 seconds, produced from 0 to 5 petechiae. At −200 mm. Hg, no petechiae were produced. Even at −350 to −400 mm. Hg, the number of petechiae was usually less than 15, indicating a resistance of the vascular wall.

Under high power, the blood vessel wall at the site of a petechia appeared to be intact and devoid of hemostatic platelet plugs, indicating that petechial formation occurred by diapedesis. A distinction should be made, therefore, between the susceptibility to petechial formation as measured by the negative pressure test and the tendency of the vascular wall to rupture (rhexis). The strength of the vascular wall resisting hemorrhagic rupture is being tested by the use of graded electric shocks from a microelectrode applied to the wall with a micromanipulator (Fulton, Akers and Lutz, 1952). The petechiae were located at the junctions of small venules with larger venules. The points of junction appeared to be vulnerable. No petechiae were formed on the capillaries hence the term "capillary fragility" is not applicable.

In the adrenalectomized hamster from 1 to 3 days after operation, susceptibility to petechial formation was definitely increased as indicated by 15 to 40 petechiae at −300 mm. Hg in most of the animals (10 of 21 examined). In the advanced stages of adrenal insufficiency at 5 to 7 days, the vascular walls were extremely resistant to petechial formation, none being formed at −400 to −450 mm. Hg. This reversal beginning at the third day was paralleled by an increase in the amount of vasoconstriction. In the starved hamsters with intact adrenals, vasoconstriction was present terminally and was also accompanied by an increased resistance of the vascular walls to petechial formation.

Susceptibility to White Thromboembolism. Using a method devised by Pierce, Lutz and Fulton (to be published), we applied dilutions of a standard solution of thrombin topically to eight adrenalectomized hamsters, as a test for susceptibility to the formation of white thromboembolism (intravascular platelet and leukocytic aggregates). In normal hamsters, thromboembolism occurred generally throughout the venules following application of 2.5 to 5.0 per cent dilutions. Adrenalectomized hamsters were within the normal range.

As an additional test for thromboembolism, a microelectrode was used to deliver injurious electric shocks directly to the blood-vessel walls. In normal hamsters, platelet thromboembolism occurred in arterioles and venules with single shocks of 4 to 6 volts of 10 milliseconds duration. The adrenalectomized hamsters were within the normal range.

The thrombin test and the electrical test may not necessarily measure the same properties, the results with thrombin probably being determined by the chemical reactions in coagulation, while the electrical test may indicate the susceptibility of the endothelium to damage or changes in platelet agglutinative properties.

The blood counts in four adrenalectomized hamsters at five days after operation were essentially within the normal range, although the red cell counts in three animals were slightly elevated, indicating a possible hemoconcentration.

Reactions During Stress. Lethal total-body x-irradiation (1200 r.) was given to 10 hamsters, immediately following bilateral adrenalectomy. Death occurred at 4 to 5 days, in contrast with an expectancy of 9 days in the irradiated hamster with intact adrenals and 5 to 7 days after adrenalectomy alone. Marked vasodilation occurred in the cheek pouch. More extensive petechial formation resulted from negative pressure than in nonirradiated adrenalectomized hamsters. The degrees of vasodilation and decreased resistance to petechial formation were comparable to that of irradiated hamsters with intact adrenals. These findings imply that a decrease in vascular resistance may occur in response to a stressing agent during adrenal insufficiency.

Preliminary observations have been made on the inflammatory response in the cheek pouch to the subepithelial injection of minute amounts of five per cent formalin. The adrenalectomized hamster appears to react more severely than the controls. Further work is in progress on the mechanisms involved in this hypersensitivity.

Vascular Effects of Cortisone Acetate

In ten normal hamsters, 10 mg. of cortisone acetate* was given subcutaneously, each day for 21 days or until death. A second series of ten normal hamsters was given 5 mg. each of cortisone daily for 21 days. In four hamsters, 15 mg. was injected daily. All four of the latter died after 12 to 21 days. Treatment with 5 or 10 mg. produced death in 14 to 30 days. In contrast with hamsters treated with DCA and salt, those on cortisone consumed food until one or two days prior to death. In terminal stages, the hamsters lost considerable weight and some hair was shed.

The small blood vessels in the everted cheek pouch were inspected at daily intervals for significant effects, and all of the tests described above were performed (ECG; electrical and chemical thresholds for stimulation and white thromboembolism; petechial formation; hematology). Tissues were fixed for histological study.

Vasoconstriction. The arterioles in the cheek pouch were constricted in all hamsters treated with cortisone (24 animals). Although "rapid vasomotion" was observed at times (in 8 animals), the arteriolar smooth muscle usually remained in a state of firm contraction, from around the seventh day of treatment until death (PLATE 2, FIGURES C and D). The precapillary sphincters were especially narrow.

The threshold of the arterioles to vasoconstrictor substances, determined by topical applications of nor-epinephrine, varied from 1:900,000 to 1:1,500,000 in the 12 hamsters that were tested. This indicates a decreased sensitivity, when compared with the threshold range for the normal hamster of 1:1,500,000 to 1:2,500,000. The threshold of the vascular smooth muscle to electrical stimulation, determined by application of a microelectrode to the blood-vessel wall, was slightly higher than that of the normal hamster and definitely increased over the threshold of the adrenalectomized hamster (observed in 6 of 11 animals examined).

No clear-cut report of a vasoconstrictor effect of cortisone has been found in the literature. Vasoconstriction resulting from cortisone administration might explain a number of clinical findings. For example, Dr. R. G. Desai (personal communication) has noted a decreased bleeding tendency in patients treated with cortisone. This hemostatic effect of cortisone might well be explained on the basis of vasoconstriction.

Diminished Petechial Formation. During cortisone treatment, the blood vessels in the cheek pouch were resistant to the application of negative pressure as a test for petechial formation. After treatment with 5 mg. of cortisone daily for 13 days, the application of −300 mm. Hg for 60 seconds produced relatively few petechiae and, after 15 to 22 days of treatment with either 5, 10, or 15 mg. of cortisone daily, no petechiae were produced by the application of from −300 to −400 mm. Hg (10 animals examined).

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* "Cortone," kindly supplied by Dr. Elmer Alpert, Merck and Company.

The resistance to petechial formation is probably due to the vasoconstrictor effect of cortisone. Shulman and Fulton (unpublished data) have found a correlation between susceptibility to petechial formation as determined by application of negative pressure and the state of the vascular smooth muscle. In hamsters with erythematous cheek pouches, within 72 hours after lethal total body x-irradiation, the application of negative pressure produced large numbers of prominent petechiae, but in hamsters with pale cheek pouches produced by Adrenoxyl 500, very few petechiae developed. Apparently, resistance decreases with vasodilation and increases with vasoconstriction. Furthermore, administration of cortisone after adrenalectomy produced greater vasoconstriction in the cheek pouch than that occurring in untreated adrenalectomized hamsters. This was correlated with an increased resistance to negative pressure, particularly during the first few postoperative days, during which the resistance to petechial formation of untreated adrenalectomized hamsters is diminished.

Robson and Duthie (1950) reported that ACTH produced a rise in "capillary" resistance and that exogenous adrenalin produced a similar effect, interpreted as due to the liberation of ACTH by action of the adrenalin on the anterior lobe of the pituitary. It is probable that adrenalin *per se* would increase vascular resistance because of vasoconstriction, and that ACTH might act by releasing cortisone. In preliminary experiments, two hamsters were injected subcutaneously with daily doses of 1.5 mg. ACTH (3 injections of 0.5 mg.) and two received 15 mg. daily (3 injections of 5 mg.) for three weeks. Marked vasoconstriction was observed in all 4 hamsters. Further work with ACTH is in progress.

White Thromboembolism. After prolonged treatment with cortisone (15 to 20 days), leukocytes were observed in large numbers sticking to the endothelial walls of venules in the cheek pouch (in 11 of 24 animals examined; PLATE 2, FIGURE E). Mixed platelet and leukocytic coatings lining the small blood vessels have been observed in the cheek pouch of hamsters subjected to infection (*Staphylococcus aureus*), malignant neoplasia, or irradiation (Lutz, Fulton, and Akers, 1951; Fulton, Lutz, Joftes, and Maynard, 1952). Lutz, Fulton, and Akers (1951) suggest that such linings in the small blood vessels of vital organs, such as the brain, heart, and lungs, might interfere with the oxygen, carbon dioxide, and nutrient exchange between blood and tisses, thus accounting for various manifestations, such as central nervous disturbances, during severe infections. A similar argument could apply to the late stages of cortisone poisoning. The prevalence of these leukocytic coatings in a growing variety of injurious circumstances indicates that they are part of a non-specific response to damage.

The threshold for white thromboembolism as tested by topical application of thrombin solutions was lowered over the normal value (in 11 of 14 animals tested). A dilution of 1.25 per cent produced generalized thromboembolism in 11 of 14 cortisone-treated hamsters, as compared with 2.5 to 5.0 per cent required normally. As tested by stimulation of the venules with a microelectrode, the threshold for white thromboembolism appeared to be elevated (from 5–7 to 6–10 volts, in 4 of 6 animals tested). This may be due to a resistance of the endothelium to damage, paralleling the resistance to petechial formation with negative pressure.

A leukocytosis occurred near death in all cortisone-treated hamsters (8), which were examined hematologically. The total white-blood-cell count ranged from 8,000 to 52,000 per cu.mm., as compared with the normal range of 3,300 to 9,600. Furthermore, the differential white-cell count was reversed over that of untreated hamsters, because of a relative lymphocytopenia. After cortisone administration, the values for the differential count were 90 to 97 per cent polymorphonuclear leukocytes, and 3 to 8 per cent lymphocytes. These findings are similar to those reported by Lutz, Fulton, and Akers (1951) during infection of hamsters with *S. aureus*. In untreated hamsters, the values were 14 to 35 per cent polymorphonuclear leukocytes, and 56 to 82 per cent lymphocytes. The blood platelets were increased in numbers in 6 of the 8 hamsters examined. In terminal stages, the erythrocyte counts and the hemoglobin values were moderately low. In 5 of the 8 animals examined the red count was less than 6 million and in one it was around 3 million (normal, 5.5 to 8.5 million), and the hemoglobins were from 5.5 to 9 gms. per cent (normal, 14 to 19 gms. per cent).

Circulating Aggregated Red Cells. In hamsters near death from overdosage with cortisone, the erythrocytes frequently circulated in loose aggregates, producing granular flow and sometimes a flow of clumped red cells (in 5 of 24 animals examined; PLATE 2, FIGURE F) like that seen prior to death from lethal x-irradiation (Fulton, Lutz, Joftes, and Maynard, 1952). Similar clumping of the circulating red cells has been observed in the cheek pouch of hamsters during severe infection, advanced neoplasia, or chronic treatment with urethane (see Lutz, 1951). Possibly this phenomenon, like the formation of leukocytic coatings described above, is also part of a nonspecific response to serious injury. Since the aggregates resolve into individual erythrocytes at capillary origins without occluding the vessels, the term "sludged blood" is not appropriate for this phenomenon.

Infections. In cortisone-treated hamsters, local infections developed frequently in cheek pouches at points of lesion such as the holes produced by pinning out the pouch. This occurred in 13 of the 24 hamsters after treatment for two weeks. The normal hamster is remarkably resistant to infection in the cheek pouch, and we did not observe infection in any of the adrenalectomized or DCA-treated animals.

The view that cortisone and ACTH render the organism more susceptible to infection and therefore should be used with caution is supported by a rapidly accumulating body of evidence. Moreover, the anti-inflammatory action of cortisone seems to be well established, and it is likely that the depression of this defense mechanism (inflammation) is responsible for the susceptibility to infection. There is an excellent recent review of the subject (Kass and Finland, 1951). Among numerous other recent works are reports that ACTH and cortisone suppress the phenomenon of local tissue reactivity in rabbits (Shwartzman, Schneierson, and Soffer, 1950), and that death from spontaneous bacteremia is common in cortisone-treated mice (Berlin, Johnson, Hawk, and Lawrence, 1952). At what point the corticosteroids act in the series of events ("chain reaction") involved in inflammation is not definitely determined. There is some evidence that corticosteroids have a direct influence on the defensive cell elements. Crepea, Magnin, and Seastone (1951) have reported a decrease *in vitro* of the phagocytic activity of neutrophilic leukocytes from patients treated with ACTH or cortisone. It seems likely, however, that cortisone also exerts an important influence quite early in the chain of events by delaying the transportation of materials and cellular elements to the site of cellular injury and/or reducing their availability to the tissues outside of the blood vessels. Menkin (1951) has described an inhibition of the increase in permeability of blood vessels in inflammation after cortisone and ACTH. Moon and Tershakovec (1952) have reported that diapedesis and edema resulting from thermal burns were decreased in cortisone-treated guina pigs, and Michael and Whorton (1951) found that the margination and migration of leukocytes and the formation of edema in the skin of rabbits in response to application of croton oil are delayed and reduced by cortisone treatment. Elsewhere in this monograph, Ebert and Barclay report that observations of the blood vessels in the rabbit-ear chamber show that, during infection or serum sickness, cortisone produces better maintenance of vascular tone, reduced damage to vascular endothelium, and decreased diapedesis of leukocytes and exudate formation (cited by permission of Dr. Barclay). It should be borne in mind that all blood vessels, including capillaries, are surrounded by connective-tissue coatings and that corticosteroids are thought to have an important influence on this type of tissue. Since some of the first events in inflammation are certainly concerned with blood vessels, it seems probable that the vasoconstrictor effect of cortisone as seen in our investigations would contribute importantly to the susceptibility to infection by interfering with the transportation of defensive materials and cellular elements and the delivery of them to the tissues through vascular walls.

In infected cheek pouches, spontaneous twitching of striated muscle fibers similar to that observed extensively in DCA-treated hamsters (see below), was observed occasionally (perhaps in one of every 4 or 5 infected animals).

Vascular Effects of DCA

The vascular effects of desoxycorticosterone acetate were studied in the cheek pouch at daily intervals after treatment. In preliminary experiments, four hamsters were given daily subcutaneous injections of 5 mg. of Percorten in sesame oil without salt in the drinking water, and two hamsters were given 10 mg. of Percorten* daily. Since the hamsters appeared healthy at 21 days, and since no alterations of the circulation in the cheek pouch had been seen, one per cent sodium chloride was added to the drinking water. Death occurred approximately 10 days later. DCA pellets (approximately 25 mg.) were implanted subcutaneously in the cervical region of eight hamsters, six without and two with salt. The hamsters with salt lost weight and died, while those without salt maintained their weight and lived. In order to determine a useful lethal dose of DCA with salt, four hamsters were given daily subcutaneous injections of 10 mg. Percorten Microcrystalline,* eight hamsters received 2.5 mg. daily, four received 1.0 mg. daily, and four received 1.0 mg. every other day. The treated hamsters stopped taking food from 3 to 5 days prior to death. Since 1.0 mg. daily plus salt was lethal within 10 to 20 days, a series of 24 normal hamsters were treated with this dosage and examined intensively for vascular effects. Furthermore, observations were made on the effects of small DCA pellets implanted in the cheek pouch of several hamsters.

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* This and the microcrystalline Percorten were kindly supplied by Dr. Robert Gaunt, Ciba Pharmaceutical Products, Inc.

The blood vessels appeared relatively normal until the time of decreased food intake and marked weight loss after 7 to 10 days of treatment. Ultimately, leukocytes were observed sticking to the blood vessel walls, forming linings or "pavements" (in about 70 per cent of the animals). The threshold for white thromboembolism, determined by either the topical thrombin test or the electrical test, was within the normal range.

Occasional arterioles were strongly constricted, especially in hamsters in terminal stages. Sausagelike constrictions of arterioles were seen in the cheek pouches containing implanted pellets. The extent and degree of vasoconstriction with DCA and salt was much less, however, than that obtained with cortisone. The threshold of the vascular smooth muscle as determined by topical application of nor-epinephrine and also by electrical stimulation with a microelectrode was within the normal range. Electrocardiograms were recorded in 18 hamsters but no remarkable changes were found.

Hematological studies were made near death in eight hamsters receiving 1.0 mg. DCA daily plus salt. A leukocytosis of 11,000 to 21,000 white cells per cu.mm. was present in four hamsters, and a reversal of the differential count occurred in seven hamsters. These changes in the DCA-treated hamsters were not as great as those produced by cortisone. No infections were found in the cheek pouches during DCA administration. The red-cell counts were normal and the hemoglobin values were nearly so.

Increased Petechial Formation. During treatment with DCA, DCA and salt, or DCA in pellet form, the blood vessels in the cheek pouch were more susceptible to petechial formation than in the untreated hamster (in 15 of 30 animals examined). From 6 to 10 petechiae were formed in response to 200 mm. Hg of negative pressure and 10 to 15 or more occurred at −300 mm. (PLATE 1, FIGURE D). This decreased vascular resistance persisted during the entire period of treatment, and until death in the hamsters receivng lethal overdosages. The cheek pouches remained relatively vascular during treatment, and erythematous pouches were characterized by an especially reduced resistance as determined by the negative-pressure test. Unlike the effects of cortisone, the vasoconstriction produced by DCA and salt was occasional and much less in degree and extent.

In control experiments, six normal hamsters were given one per cent sodium chloride in the drinking water. The blood vessels in the everted cheek pouch appeared normal. Application of the negative-pressure test indicated no significant changes as a result of salt intake.

Twitching of Striated Muscle. Individual striated muscle fibers in the cheek pouch exhibited intermittent spontaneous twitching in 50 per cent of the DCA-treated hamsters. This effect was present in most of the pouches containing implanted DCA pellets. At this time, we have no explanation for this phenomenon.

Summary

The blood vessels and blood flow in the everted and transillumined cheek pouch of adrenalectomized hamsters appeared relatively normal until 24 hours prior to death at 5 to 7 days. At this time, the rate of flow decreased and stasis occurred, probably resulting in part from a bradycardia and irregularities in the heart action as indicated by electrocardiograms. Improvement of the circulation occurred with cortisone and also with DCA. The arterioles in the cheek pouch became progressively constricted from 3 to 4 days after adrenalectomy until death. "Rapid vasomotion," which has not been seen in the cheek pouch of normal hamsters, occurred in a few animals. The sensitivity of the smooth muscle to direct electrical stimulation with a microelectrode was increased as compared with normal values, but the sensitivity to topically applied nor-epinephrine was decreased. Although spontaneous petechiae were rarely found, the susceptibility of the venules to petechial formation in response to negative pressure from a suction cup was greater than normal during the first three postoperative days. In advanced stages of insufficiency, the vascular walls were resistant to petechial formation, paralleling the occurrence of vasoconstriction.

In intact hamsters receiving 5 or 10 mg. of cortisone acetate daily, arterial vasoconstriction predominated in the cheek pouch, from an early period until death after 14 to 30 days. Occasional "rapid vasomotion" was observed. The sensitivity of the vascular smooth muscle was decreased as indicated by the response to nor-epinephrine and also to stimulation with a microelectrode. The resistance of the blood vessels to petechial formation as a result of negative pressure was increased. Susceptibility to white thromembolism formation in response to electrical damage of the endothelium was decreased. The prolonged vasoconstriction accompanied by decreased sensitivity to stimulation and increased resistance to mechanical or electrical damage of the vessel walls may account for certain clinical findings, such as a decreased bleeding tendency in patients treated with cortisone. Furthermore, the vasoconstriction may help to explain the anti-inflammatory action of corticosteroids and the increased susceptibility to infection that may result from their administration. Infections of the cheek pouch of cortisone-treated hamsters were common. After extended cortisone treatment, leukocytes were observed lining the walls of venules, and the erythrocytes sometimes circulated in loose aggregates shortly before death. These phenomena may be nonspecific responses to injury. There was a terminal leukocytosis and a reversal of the polymorphonuclear–lymphocyte ratio (due to a relative lymphocytopenia). White thromboemboli were formed more readily than in the normal hamster in response to the topical application of thrombin solutions.

In intact hamsters receiving 1.0 mg. of DCA daily, together with one per cent saline drinking water, the blood vessels of the cheek pouch appeared relatively normal until marked weight loss occurred prior to death. Although vasoconstriction was observed occasionally, it was not comparable to that produced by cortisone. Decreased resistance to negative pressure with increased petechial formation persisted throughout the period of treatment. Since the pouches were relatively vascular, this confirms the conclusion that resistance to petechial formation is increased by vasoconstriction and decreased by vasodilation. Leukocytic coatings in the small vessels, a moderate leukocytosis, and reversal of the differential count were observed in the terminal stages.

Intermittent spontaneous twitching of striated muscle fibers in the cheek pouch occurred in 50 per cent of the DCA-treated hamsters and in some of the infected pouches of cortisone-treated animals.

A motion picture (Kodachrome, 16 mm.) of significant findings in adrenal insufficiency and experimental hypercorticalism has been prepared.

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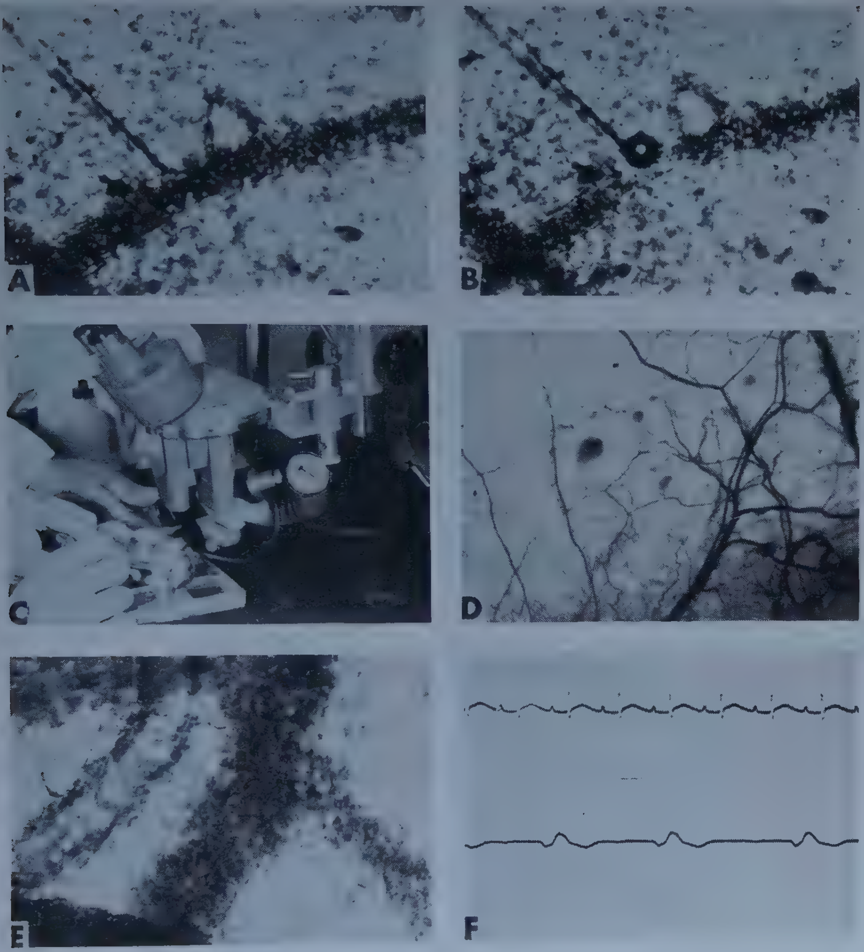

Plate 1

Figures A and B. Stimulation of an arteriole with a microelectrode, producing vasoconstriction, as a test for sensitivity of vascular muscle. Original magnification, × 200. Figure A, before stimulation; figure B after stimulation (bubble at tip of microelectrode indicates moment of stimulation).

Figure C. Application of suction cup to the cheek pouch as a test for susceptibility to petechial formation. Dial gauge indicates negative pressure.

Figure D. Petechial formation at 250 mm. Hg negative pressure after DCA and salt treatment. Original magnification, × 7.

Figure E. Slow flow and stasis 7 days after adrenalectomy. Original magnification, × 200. Venule packed with slowly moving cells.

Figure F. Electrocardiogram of normal hamster (upper record) compared with that of an adrenalectomized hamster (lower record), 4 days after operation. Bradycardia and abnormal QRST complex.

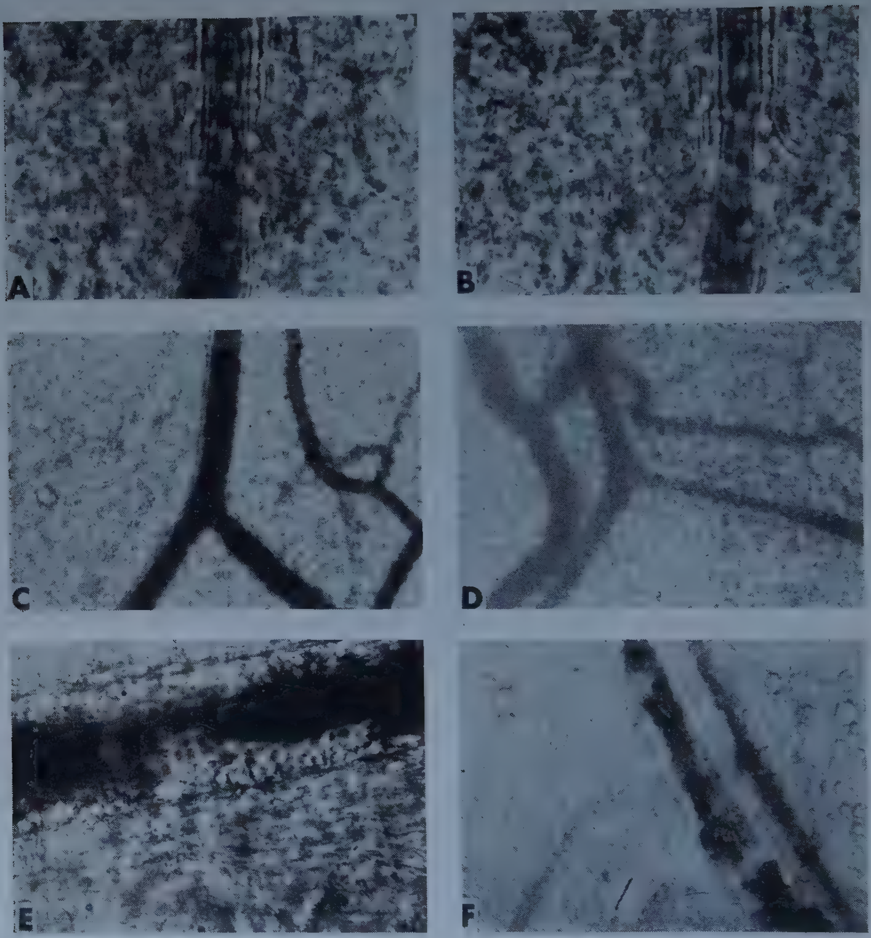

Plate 2

Figures A and B. "Rapid vasomotion" of an arteriole 6 days after adrenalectomy. Original magnification, × 440. Figure A, dilator phase; figure B, constrictor phase.

Figures C and D. Cheek-pouch vessels of a normal hamster (figure C) compared with those of a hamster after 9 days of treatment with cortisone acetate (figure D). Original magnification, × 100. Figure D shows chronic arteriolar constriction produced by cortisone treatment.

Figure E. Intravascular leukocytic coating lining the vessel, as seen after prolonged treatment with cortisone acetate or DCA and salt. Original magnification, × 260.

Figure F. Aggregation of circulating red cells as seen shortly before death from overdosage with cortisone acetate. Original magnification, × 100.

HEAD BLOOD FLOW, OXYGEN CONSUMPTION, AND ELECTRICAL ACTIVITY IN RELATION TO THE ADRENAL CORTEX*

By Hudson Hoagland, John R. Bergen, Alan Slocombe, and Charlotte Hunt
The Worcester Foundation for Experimental Biology, Shrewsbury, Mass.; and the National Institute of Mental Health Cooperative Research Station at the Worcester Foundation, Public Health Service, Federal Security Agency

A number of studies have indicated that steroid hormones may modify the behavior of the nervous system. Thus, adrenocortical function has been related to psychosis,[1, 2, 3, 4, 5, 6] to aspects of fatigability,[7, 8] and to modifications of the electroencephalogram (EEG) in Addisonian patients.[9] Several reviews of these matters have appeared, but very little is known as to the manner in which steroids may affect events in the nervous system. The following experiments were undertaken to shed light on this problem. The light so far is little more than a faint glow, but some of the leads are suggestive in terms of developing investigations.

The EEG's of Addisonian patients are abnormally slow and can be brought to normal by the administration of cortisone.[9] Several years ago, in our laboratory, Bergen[10] demonstrated a slowing of the EEG in the unanesthetized, adrenalectomized, and salt-maintained rat. The EEG in the normal unanesthetized rat is reduced from a median frequency of 44 waves per second to 38 following adrenalectomy. We found that an injection of 0.5 cc. of Upjohn's lipoadrenal extract (ACE), equivalent to 0.5 mg. compound F, fully restores the EEG to normal three hours after injection. Similar restoration of the EEG three hours post-injection is brought about by 0.25 mg. of Δ-5-pregnenolone, although desoxycorticosterone (DOC) in twice this dose has no effect in increasing the EEG frequency. Other steroids have not yet been investigated.

Slowing of regular frequencies in the EEG may be a sign of decreased oxygen and carbohydrate metabolism of the brain.[11, 12] It has been demonstrated independently by Gordan *et al.*[13] and by Hayano and Dorfman[14] that certain steroids in relatively large amounts inhibit the oxygen consumption of ratbrain homogenates through action on specific flavo-protein entities of the electron-transfer system. We have measured the oxygen consumption in Warburg manometers of rat-brain homogenates and cerebral-cortex slices taken from normal and from adrenalectomized rats but have found no group differences whatever in the oxygen utilization of brain. We have modified the glucose content of the medium and studied effects of temperature on oxygen consumption, but here, too, we have not found these factors to differentiate in oxygen consumption between the two groups. The activation energy for oxygen consumption of rat brain determined by the Arrhenius equation is the same in both groups over a temperature range of 25 to 37°C. We

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have assayed the brains for cytochrome oxidase and succinic dehydrogenase and again have found no differences in the titers in brains from normal and adrenalectomized rats. Our *in vitro* studies of oxygen consumption within the physiological range have thus yielded entirely negative results.

Despite these findings *in vitro*, it could be nevertheless that slowed *in vivo* oxygen consumption might account for the decreased frequency of the EEG in the adrenalectomized animals. Levine *et al.*[15] have demonstrated that ACE but not DOC acts on the circulation of adrenalectomized animals to elevate depressed blood pressure and thus enhances muscle work output *in vivo*. It seemed possible, therefore, that the slowed EEG of adrenalectomized animals kept in good condition on salt might arise from slowed cerebral circulation and accompanying oxygen deprivation.

To test this, the rate of blood flow from the left posterior facial vein of the rat draining the brain sinuses was measured after ligating a number of other veins. The relative rate of blood flow was determined as follows: The rats were anesthetized with nembutal and an incision made from the tip of the sternum to the mandible. By means of blunt dissection, the external jugular veins of both sides were traced to their origins, *i.e.* the junction of the anterior and posterior facial veins. According to Greene,[16] the posterior facial veins form the main exit of blood from the transverse sinus of the brain via the internal maxillary veins. Although a number of other veins draining the head region empty into the posterior facial vein, the anterior and posterior facial veins of one side were clamped and the anterior facial vein of the opposite side ligated. Branches draining into the remaining posterior facial vein were ligated or clamped in order to reduce flow from branches of veins draining muscles as much as possible. Thus, the veins draining the pterygoid and pharyngeal plexuses and the vein from the masseter muscle were tied off. The posterior auricular vein was routinely stretched and broken with no subsequent blood flow from this vessel. The remaining large branches of the posterior facial vein are the internal maxillary and the superficial temporal veins.

The internal maxillary vein drains the vessel from the transverse sinus and receives an articular vein, the posterior deep temporal vein, and a communicating vein from the inferior alveolar vein. Extensive surgery would be required to tie off all the branches of the internal maxillary, excepting the transverse sinus vessel, so these branches were left intact. Immediately before collecting blood from the remaining posterior facial vein, the external jugular vein on the same side was ligated, preventing any back flow of blood through this channel. The left external jugular vein now supplied mainly by the posterior facial vein was severed above the ligature and the blood was collected for one minute on tarred cotton swabs. Immediately afterward, another sample was taken for hemoglobin determination. The quantity of blood flowing from the severed vessel was then determined by its weight and also calculated from the total hemoglobin content of the collected blood determined as alkaline hematin. No difference in hemoglobin content has been found in blood from control and adrenalectomized rats.

The oxygen determinations were made according to the Roughton-Scholander method from blood samples taken from the common carotid artery and from the posterior facial vein prepared as above. Blood flow determinations and the arteriovenous oxygen difference could not be determined on the same rat. Since we do not know the proportion of blood in our samples coming from the brain sinuses and from other tissues of the head, we refer to the head blood flow rather than brain blood flow. That the head blood flow and oxygen consumption is probably proportional to brain blood flow is, however, indicated by our data relating these variables to the frequency of the ECG.

We found a reduction in head blood flow of 61 per cent in adrenalectomized rats and a corresponding decrease in tissue oxygen consumption of 43 per cent, which are highly significant differences statistically. While a relatively greater percentage of oxygen is removed by the tissues from the slowly flowing blood in the adrenalectomized rats, the total reduction in blood flow is sufficiently great to reduce the tissue's oxygen supply substantially. TABLE 1 shows data illustrating the differences in blood flow and oxygen consumption in normal and ardenalectomized rats and in those given replacement therapy.

TABLE 1

HEAD BLOOD FLOW, ARTERIAL AND VENOUS OXYGEN VALUES, AND STANDARD ERRORS IN NORMAL AND ADRENALECTOMIZED RATS MAINTAINED WITH SALINE

Rats	No. of rats	Blood flow, ml./min.	No. of rats	Arterial oxygen, vol. per cent	Venous oxygen, vol. per cent	A − V diff., vol. per cent	Oxygen consumed, ml./min.	Per cent of normal oxygen used
Normal	8	1.65 ± 0.12	13	14.2 ± 0.26	7.7 ± 0.37	6.5	0.107	—
Adrex	19	0.65 ± 0.07	9	14.8 ± 0.80	5.4 ± 0.89	9.4	0.061	57
ACE treated	17	1.69 ± 0.14	8	13.4 ± 0.58	7.9 ± 0.60	5.5	0.093	86
Pregnenolone treated	13	1.34 ± 0.11	11	14.2 ± 0.60	6.9 ± 0.79	7.3	0.098	92
DOC treated	16	1.05 ± 0.15	16	14.6 ± 0.32	8.3 ± 0.47	6.3	0.066	61

The administration of 0.25 ml. (equivalent to 0.25 mg. of compound F) of Upjohn's lipoadrenal extract to adrenalectomized rats restored completely in two to three hours the blood flow, which averaged before injection only 39 per cent of its normal flow. We have found that 0.25 mg. of Δ-5-pregnenolone restored blood flow to 81 per cent of normal, but DOC even in twice this dose raised it to only 61 per cent of the normal flow. Cortisone (0.25 mg.) restored head blood flow to normal in two to three hours. The steroids also effect oxygen consumption, as may be seen from the table. The tissue oxygen consumption of the adrenalectomized rats is reduced to 57 per cent of its normal value. ACE restores it to 86 per cent. The restoration is 100 per cent in this case, if arterial oxygen is assigned a value of 14.2 volumes per cent. It may be seen from TABLE 1 that arterial oxygen values range from 14.2 to 14.8 volumes per cent in four sets of experiments on 49 rats. In the ACE study the low mean value of 13.4 was obtained on 8 rats. At the time of this particular study, our colony was experiencing a respiratory infection, which may account for this atypical value.

The effects of ACE, of pregnenolone, and of DOC on blood flow and oxygen consumption parallel the effects on the EEG. Figure 1 shows data summarizing the per cent changes in blood flow, glucose utilization, oxygen consumption, and EEG frequencies. The height of the first set of four rectangles

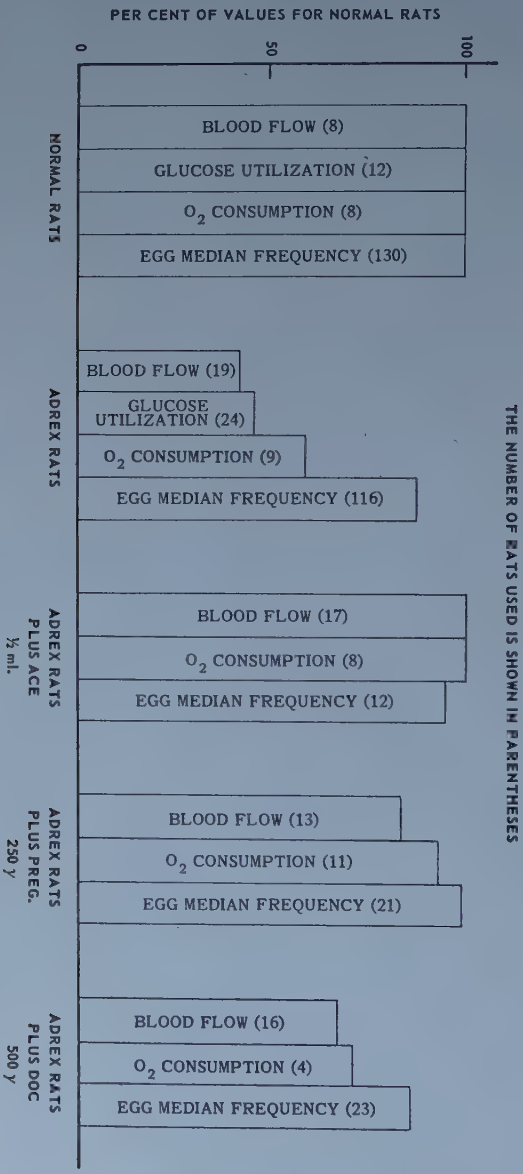

Figure 1. Effects of adrenalectomy and replacement therapy on head blood flow, oxygen and glucose consumption, and on the frequency of the EEG.

are assigned values of 100 per cent, and comparative changes following adrenalectomy and replacement therapy are shown in the blocks of rectangles to the right. We have been especially interested in the effects of Δ-5-pregnenolone on the central nervous system, in view of our earlier observations of its antifatigue action in man.[7, 8]

From these findings, we have concluded that the changes in the EEG following adrenalectomy and their restoration with certain steroids probably result from the effects of these manipulations on cerebral blood flow and oxygen consumption. Our negative *in vitro* studies strongly suggest that the steroids play no direct role in the actual oxygen consumption of brain tissue *per se.* Reduced head circulation in adrenalectomized animals appears to decrease brain oxygen consumption, which slows the EEG.

Effects of Adrenal Steroids on Synaptic Conduction and Nerve Excitability. We have extended our investigations to other aspects of nervous-system function. Slocombe has studied the synaptic delays of impulses conducted from the foot to the somatic sensory cortex of the rat. The animals are anesthetized with pentathol and holes are trephined through the skull for recording purposes. Because the "spontaneous" activity of the cortex (EEG) interferes with the somatic response, it has been necessary to use a level of anesthesia that just eliminates this activity and leaves the cortical response clear and reproducible. This requires considerable depth of anesthesia in the rat, which must be controlled with some precision. Slocombe has used a modification of the Bickford servoanesthetizer to control depth of anesthesia. The rat's EEG determines the rate of injection of the anesthetic into the femoral vein. The apparatus is set so that, with a disappearance of the EEG, the anesthetic is turned off; with its reappearance, further injection is automatically produced. With this control of depth of anesthesia, the rate of conduction from foot to somatic sensory cortex in normal and in adrenalectomized rats kept in good condition on salt has been determined. TABLE 2 shows a slowing of 18 per cent in conduction from foot to cortex in the adrenalectomized animals studied seven days post-operatively. Rectal temperatures of all rats were recorded, and the measurements were made at normal body temperature, obtained in some cases by warming with a heating pad.

TABLE 2
CONDUCTION TIME FOOT TO SOMATIC SENSORY CORTEX
(Temperature 98.5–99.4°F)

	Number of animals	Cond. time (ms.) & S.E.	Per cent difference above normal	*t*	*P*
Normal rats	20	11.3 + 0.3	—	—	—
Adrex. rats	14	13.3 + 0.4	18.0	4.0	<0.001
Adrex. rats given ACE	14	12.2 + 0.3	8.0	2.1	<0.025
Adrex. rats given cortisone	13	11.3 + 0.2	0.0	—	Not different from normal

Conduction time of the cortical response is 11.3 ms. in normal rats and 13.3

ms. in adrenalectomized rats. The third line of TABLE 2 shows that 1.1 cc. of lipoadrenal extract (equivalent to 1.1 mg. of compound F), administered in four divided doses at 22, 6, 4, and 2 hours before the test, reduces the delay of conduction in adrenalectomized rats from 18 to 8 per cent. The use of 1.1 mg. of cortisone administered at the same time intervals fully restores the conduction time (fourth line of TABLE 2).

We have also examined the excitability of the rat sciatic nerve *in situ* by measuring Hill's excitation constant.[17] This is the time constant of the rate at which, after accommodation to the stimulating current, the nerve threshold reverts to its original level. It is thus a basic measure of the electrical properties of the tissue. The time constant is increased by 146 per cent following adrenalectomy (mean of 7 intact and 11 adrenalectomized rats). Three hours after the injection of 1.1 cc. of lipoadrenal extract, the time constant is reduced to 87 per cent above normal, and these differences are statistically significant.

Discussion

The foregoing work raises more questions than it answers. Our *in vivo* studies indicate that adrenal cortical hormones modify the timing of impulses in the nervous system as indicated by modification of the circuits engaged with the EEG and in measures of conduction time in the central nervous system. We have also presented evidence indicating that the excitation of the sciatic nerve in the intact animal is decreased by adrenalectomy and partially restored by ACE. This extract fully restores decreased brain circulation and oxygen consumption and the EEG frequency in three hours. It restores only partially conduction time, even when administered over a longer period and in a larger total dose. It also restores only partially the decreased nerve excitability three hours post-injection. While modifications of the circulation would appear to account for the slowing of the EEG by reducing the oxygen consumed by the brain, the less effective restoration of conduction time and of somatic excitability by ACE may involve other factors.

At present, we are engaged in testing specific steroids in terms of the relations between their chemical configurations and how they affect circulation, oxygen consumption, and conduction. We are comparing equal doses of cortisone and compound F. We wish, for example, to compare effects of the presence or absence of hydroxyl at the C-21 position in compound F and 21-desoxy F. Comparisons of progesterone with pregnenolone are also planned. Eventually it may be possible to characterize the effects of details of molecular steroid structure in terms of their action on events in the nervous system.

Summary

We find no *in vitro* differences in oxygen consumption of brain homogenates and slices from normal and adrenalectomized rats. The slowed EEG of the rat following adrenalectomy is restored by ACE and by pregnenolone but not by DOC. Blood flow through the head of adrenalectomized rats is markedly reduced, as is the tissue oxygen consumption. ACE, Δ-5-pregnenolone,

but not DOC, in dosages comparable to those affecting the EEG, also restore head blood flow and oxygen consumption. Cortisone also is effective in restoring blood flow. Its effects on the rat EEG have not been studied, although it does restore the slowed EEG in Addisonian patients.

Conduction from foot to somatic sensory cortex is decreased 18 per cent after adrenalectomy. Cortisone, fully, and ACE, less effectively, have been found to restore the conduction rate. The excitability of the rat sciatic nerve *in situ* is reduced by adrenalectomy and is partially restored by ACE.

These studies are preliminary to a systematic investigation of the role of steroid structure in affecting physiological events in the nervous system.

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THE INFLUENCE OF CORTISONE UPON GRANULATION TISSUE AND ITS SYNERGISM AND ANTAGONISM TO OTHER HORMONES*

By Matthew Taubenhaus

Michael Reese Hospital, Chicago, Illinois

The mechanisms of the inhibitory action of cortisone upon granulation tissue remain unknown. An approach towards the study of the complex problem would be, in the author's opinion, a careful description of the known facts referring to the mode of its action, its position in relation to the action of other hormones, and a correlation of its influence on granulation tissue with other known functions of this steroid. The data referring to the specific influence of cortisone upon the known facts of the physiology and biochemistry of connective tissues are as yet insufficient to permit final conclusions as to the mechanisms of its action.

The effect of cortisone on mesenchymal structures can be well observed experimentally in animals in which tissue repair occurs after various types of injury. Granulation tissue, forming in the vicinity of wounds, or chemically induced tissue necrosis appears to be markedly inhibited by that steroid.[1, 2] This inhibition becomes apparent in the first stages of inflammation,[3, 4] and cannot be observed if cortisone is administered once granulation tissue has developed,[3] although clinical observations in cases of fully developed rheumatic diseases seem to point to the contrary. The inhibitory effect of cortisone it seems, is absent in dogs in which linear wounds are healing *per primam*,[5] which suggests that some more penetrating agent (bacterial, toxic, chemical) or actual tissue necrosis must be present in order to enhance the inhibitory effect of this substance.

All the elements of granulation tissue seem to be profoundly affected by cortisone. Of the cellular elements, the fibroblast shows a very profound change.[1, 2] It appears smaller and flatter, and its nuclei seem more pycnotic. The number of fibroblasts is considerably diminished. Surrounding the injury, areas can be observed where fibroblasts and granulation tissue as a whole are absent. This appears of importance because the fibroblast has been brought in connection with the mucopolysaccharides of ground substance and has been considered to be the source of fibers, either directly or indirectly. Thus, any agent interfering with the growth of fibroblasts would necessarily influence the noncellular structures of mesenchyma.

Still under the influence of cortisone, the fibroblasts retain their orderly parallel arrangement in further stages of healing. This appears remarkable because, as will be discussed later, desoxycorticosterone profoundly interferes with this particular phase of healing. The influence of cortisone upon fibroblasts growing *in vitro* was also studied[7, 7a] but according to recent investigations, no influence could be established.[8]

In our original work on this subject,[2] large amounts of macrophages were

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observed in granulation tissue developing under the influence of cortisone. In contrast to this finding, other investigators[3, 8] found that the number of macrophages in such tissues decreased. We have, therefore, recently searched granulation tissue in a larger series of our experimental animals for macrophages. A stain that seems to spot macrophages more or less selectively was used in these experiments, which were performed through the courtesy of Dr. G. Gomori.[8a] Indeed, there were less macrophages present, which would confirm the findings of the other authors. We are unable to explain the difference between our recent and previous observations. The effect of cortisone upon lymphocytes[9] cannot be recognized in granulation tissue under these experimental conditions.

Much interest has centered upon the ground substance of connective tissues. Mainly through the work of Gersh,[10] C. Meyer[11] and others, its chemistry and physiology has been clarified to an extent. A discussion of this problem will be carried here in so far as it is related to cortisone and other hormones. We[2] have observed that, in granulation tissue developing under the influence of cortisone, the intercellular material appeared to have a structure different from the controls. Preparations stained with the Hotchkiss-McManus technique did not yield more information. Recently, it has been observed that less sulfuric acid is utilized for the synthesis of chondroitin sulfuric acid under the influence of cortisone.[12] Whether this is etiologically significant remains to be proven. It might be another expression of the fact that less of the material is formed, together with less granulation tissue. An analogy might be the observation, recently confirmed by our group, that less phosphatase is found by the Gomori technique in granulation tissue formed under the influence of cortisone. This indicates by no means a primary action of cortisone upon phosphatase activity. In conclusion, our knowledge of the influence of cortisone upon the ground substance in granulation tissue is sparse, although observations of its action upon the hexosamines of the plasma[13] and the known activity of hyaluronidase and its substrates (not further discussed here) suggest that such an influence exists. More investigative work will have to be performed to elucidate this interesting question.

It has been shown that other hormones produce changes in the chemical and physical estrogenic structure of the intercellular substance. Gonadotrophins exert a profound influence on the polymerization of the mucopolysaccharides of the ground substance in certain tissues,[4] and testosterone promotes the formation of such components in the cock's comb.[15] Thyrotropic hormone changes the hexosamine content of the retrobulbar tissue,[16] and thyroxin prevents intercellular material from becoming myxedematous.[2]

The effect of cortisone upon collagen fibers is a profound one, and concerns their growth, their cohesion, and their formation. It was observed[17] that adrenocortical extracts applied topically upon the skin of rabbits alters the fibrillar structure of cutaneous collagen lying underneath. We have found[18] in a case of scleroderma that the pathologically thickened collagen bundles become looser and assume a wavy appearance, resembling normal cutaneous tissue. These observations led us to the assumption that, under the influence of cortisone, the cohesion of collagen bundles and fibers diminished. The forces keeping the collagen fibers and fibrils together side to side are not known. A cement substance, which surrounds the fibrils and is visible on electronmicrographs, cannot be the sole agent, because collagen fibrils can be washed free of this substance and still maintain a close alignment next to each other with one axial repeating period next to the one of the neighboring fibril. We[19] could not find any difference in this alignment in electronmicroscopic studies on tail tendons of rats with or without cortisone. If the forces operating to keep fibrils together were known, an interesting effect of cortisone could be understood. In granulation tissue surrounding turpentine abscesses, collagen fibers have been sparser than in the control tissues.[2] The question as to the site of fiber formation has been discussed by many investigators,[20] and more knowledge of the mechanism of collagen-fiber formation is necessary to evaluate the mechanism of the action of cortisone thereon.

The longitudinal growth of collagen fibers is necessarily inhibited under the action of cortisone, because the steroid inhibits growth in general. It became evident from studies on collagen fibers of the skin[21] and our investigation on tail tendon fibers of the rat[19] that longitudinal growth proceeds without changes in the length of new axial repeating periods in each fibril. The length of each such period remains rather constant in young or old animals, although its width increases. In cortisone-treated animals, in which the body and tail length remain small, as compared to untreated, growing control animals, the length of the axial repeating periods of the tail-tendon fibrils remains the same as in the young and the grownup controls. The widths, however, increase, and their measurements equal, or even exceed the ones found in the adult control group.[19] It was concluded from these experiments that, under the influence of cortisone, the apposition of axial repeating periods is inhibited. The width of each fibril increases with the general growth, as in the control animals. At the present time, no information is available that would enable us even to postulate the mechanics of action of cortisone on these factors. Anterior pituitary growth hormone, on the contrary, has the opposite effect upon collagen fibers by increasing their number.[22]

Whatever the effects of cortisone on the body as a whole, the site of action upon granulation tissue and connective tissue in general is a local one. This was shown[17] by observing changes in connective tissue underlying the area where adrenal extracts were applied or by observing if granulation tissue developed in the vicinity of cortisone.[23, 24] We[23] have demonstrated that a turpentine–cortisone suspension injected into a rat will inhibit granulation-tissue formation surrounding this abscess, whereas pure turpentine injected into the contralateral side of the same animal will produce a normal granulation response. Numerous clinical observations, particularly in the fields of ophthalmology and rheumatology, point to the same assumption. This is an important observation, because it indicates that the action of cortisone differs from many other steroids, as will be discussed later.

From previous work, it became evident that cortisone is by no means the only hormone influencing granulation tissue. Among the adrenal steroids, desoxycorticosterone has an important place. The general stimulating effect upon fibroblastic proliferation in various organs and structures had been clearly demonstrated in extensive studies by Selye and his group. In order to produce such changes with desoxycorticosterone, the experimental animals had to be injected for a rather long period of time. We[25] have shown that granulation tissue is also considerably stimulated if the experimental animals were pretreated with this steroid. The structure of such tissue undergoes quite characteristic changes, consisting in enlargement and stellate shape of the fibroblasts, less fiber formation, possible changes in the ground substance, and the lack of an orderly parallel arrangement of the cellular elements such as is observed in control animals. These observations were essentially confirmed,[26] but it was pointed out that, in spite of the proliferation of granulation tissue under cortisone, wound healing is retarded. Desoxycorticosterone has an inhibitory effect upon fibroblasts developing *in vitro*,[27] but cultures of fibroblasts may not represent a suitable medium for the study of hormonal action. Desoxycorticosterone, if injected only during the period when the abscess is developing, has no effect, or even a slight inhibitory effect upon granulation-tissue formation. This was observed by us in some unpublished experiments in which desoxycorticosterone was suspended in the turpentine and injected into the subcutaneous tissues of rats. Pretreatment over a prolonged period of time is, therefore, necessary to obtain the full stimulating effect of the steroid. In considering the action of desoxycorticosterone, one must remember particularly that this steroid is rapidly metabolized in the mammalian body and transformed into other steroid compounds. The changes observed in mesenchymal structures might very well be due, therefore, to the immediate action of some desoxycorticosterone derivatives and not to the injected material. Compound F has been insufficiently studied in regard to its action upon granulation tissue to permit definite conclusion. The observations available up to date[28] seem to indicate that, in this respect, it resembles the action of cortisone, particularly in regard to its local effect.

That neither one of the adrenal or testicular steroids is actually necessary to maintain granulation-tissue development was shown in adrenalectomized and castrated animals.[25]

The anterior pituitary gland is another important organ regulating granulation tissue development. Its removal in rats uniformly results in an extremely poor granulation tissue development in response to the injury by turpentine.[2] This is quite different from adrenalectomized animals, as mentioned before. The anterior pituitary must, therefore, possess powerful stimulating effects upon granulation tissue.

Adrencocorticotrophin, as expected, proved to be an inhibitor resembling the action of cortisone.[2] Quantitatively, however, the inhibition was not as pronounced as under cortisone. The puzzling observation that ACTH is inactive in castrated animals[29] has to be investigated further.

Anterior pituitary growth hormone restored the poorly developed granulation tissue of hypophysectomized animals to normal and, in higher doses, even stimulated it beyond normal. Under the influence of this hormone, giant fibroblasts were observed in some of the preparations. Administered to intact animals, growth hormone also exerted a stimulating effect, producing an excess proliferation of granulation tissue around the turpentine abscess.

If high doses were given to normal animals, this effect was lost, possibly because of contamination of the growth hormone preparation with other substances. Growth hormone proved inactive in adrenalectomized animals. In very extensive studies, Selye and his co-workers have demonstrated the strongly stimulating effect of growth hormone upon various mesenchymal structures, and outlined its significance in defense mechanisms against different injuries.[30]

Thyroid stimulating hormone was not applied in our experiments. An indirect test was utilized by using hypophysectomized animals and observing whether thyroxin would stimulate the lacking granulation-tissue response.[2] In such animals, thyroxin re-established a fair granulation tissue in the surrounding of turpentine abscesses, indicating that thyroid stimulating hormone enchances granulation-tissue formation in deficient animals. Thyroxin administered to intact animals had no effect and, when injected to thyroidectomized animals, caused normalization of a myxedematous granulation tissue.[31]

Estrogen causes a pronounced inhibition of granulation tissue, whether the animals were pretreated,[25] or whether it was administered simultaneously with the development of the turpentine abscess.[2] The inhibitory effect of estrogen can be counteracted by simultaneous injections of small doses of growth hormone.[2] From this, we concluded that the inhibitory action of estrogen is due to suppression of the anterior pituitary growth hormone production and/or release. A further confirmation of the systemic influences of estrogen, in contradistinction to the local one of cortisone, was gained from the following experiment: Estrogen suspended in turpentine was injected subcutaneously into one side of a rat, and turpentine alone into the other side. Small doses of estrogen had no influence on either side. As the dose was increased more and more, a pronounced inhibitory effect was observed on the turpentine–estrogen side and on the opposite side as well. This was an entirely different effect from the one obtained with cortisone if tested under comparable conditions.[24] The inhibitory effect of estrogen was confirmed by others,[32, 33, 34] but a negative effect was also reported.[29]

Testosterone had a suppressing effect similar to that of estrogen, only less pronounced. In the presence of excess growth hormone, it actually exhibited stimulation of granulation tissue. Ducommun and Ducommun,[34] who were able to confirm our findings, have also pointed out that it loses its effect in adrenalectomized animals. This is a very interesting finding, which might clarify the mode of action of testosterone. Methyl androstenediol, if injected while the turpentine abscess was developing, produced an inhibition of granulation-tissue formation similar to that of testosterone,[31] although prolonged injections of this substance into rats had a stimulating effect upon mesenchymal structures resembling that of desoxycorticosterone.[35]

The action of cortisone is synergistic with estrogen and testosterone. Nevertheless, it acts more specifically than the others because it has the unique property of influencing granulation tissue locally, without the aid of distant or systemic influences. Its inhibitory effect is opposed by growth hormone and desoxycorticosterone. In order to test this antagonism in detail, the following experiment was performed: As before, a cortisone–turpentine suspension was injected into the right side of an animal and turpentine alone was injected into the left side. Desoxycorticosterone was administered systemically to the animals used in this test for some time before and during the development of the turpentine abscesses. Quantitatively, the inhibitory effect was abolished, but the quality of granulation tissue under the influence of desoxycorticosterone was again different from the normal. The shape and the arrangement of the fibroblasts was irregular and, on the whole, the tissue looked less mature. This would indicate that desoxycorticosterone acts upon the cortisone-inhibited fibroblast, but causes its proliferation into abnormal pathways.[31] That cortisone may counteract some of the effects of desoxycorticosterone upon vascular structures was shown before.[36] In another series of experiments, hypophysectomized animals were used, and cortisone plus turpentine, and turpentine, respectively, were injected as before. The inhibitory effect of cortisone could still be observed in spite of the generally poorly developed granulation tissue. Growth hormone injected into such animals re-established normal granulation tissue. The quantity and the quality of such tissue was indistinguishable for normal control animals. This could be seen on the cortisone–turpentine and on the turpentine side as well, and would indicate that growth hormone acts locally upon the very same factors that cortisone tends to inhibit.[31]

The experiments described above would not permit more than speculations as to the mechanism of the inhibitory action of cortisone upon granulation tissue. One of its known effects is upon the catabolism of protein. Its ability to exert its influence locally, however, without affecting structures distant to its site of injection, would strongly speak against a negative nitrogen balance of the whole organism as being the cause of the granulation-tissue inhibition. An interference of cortisone with the metabolism of certain amino acids necessary for the formation of mesenchymal structures, however, has to be considered as a possible site of its action.[37] There is insufficient proof that cortisone has a primary effect upon the mucoporteins of the ground substance. Any decrease of metabolic activities in this substance may be the result rather than the cause of inhibition of granulation-tissue formation, but sufficient evidence exists to prove its profound effect upon noncellular elements of the mesenchyma. The effect of cortisone upon vascular reactivity[38] offers an approach to the understanding of its inhibitory effects. Any tissue development would be suppressed if it occurred in an ischemic medium. Cortisone then, by virtue of its locally enhancing the vasoconstricting nonadrenalin effect would interfere with new formation of tissues. In order to test this possibility, we have studied the cortisone effect upon granulation tissue in areas rendered ischemic by ligation of vessels.[24] Good granulation tissue developed in such areas, and the cortisone effect could still be observed; but these experiments are not conclusive enough to disprove the mechanism under discussion. The development of granulation tissue under the influence of autonomic-nerve-blocking agents is being studied to obtain more information on this subject.

From purely histological observations, the profound effect upon the fibroblast itself is undeniable. A suppression of its development is one of the outstanding features of cortisone action. This is the site where the growth hormone or desoxycorticosterone antagonism can be demonstrated. As long as so little is known about the relation of shape to function of this cell, no definite conclusions can be drawn. Studied demonstrating precursors of ground-substance material within the fibroblasts should be applied in relation to cortisone action.

The importance of ascorbic acid to the formation of new connective tissue, as reviewed by Ehrich,[40] its relation to the adrenal cortex, and its ability to inhibit hyaluronidase activity strongly suggest some connection between this vitamin and the inhibitory effect of cortisone upon mesenchymal structures such as granulation tissue. So far, no positive evidence for this theory has been brought forward, and we are unable to suggest any mechanism of an interaction between these two substances.

The interference of cortisone with the action of sulfhydryl systems[41] raises the question whether enzymatic processes are the primary target of cortisone action in respect to mesenchymal structures. Of particular interest is its effect upon the hyaluronidase and membrane permeability.[40] It is also interesting to speculate on the role of the electrolyte shifts occurring under steroid influence, inasmuch as it has been demonstrated that hypopotassemia will produce mesenchymal reactions recognizable histologically.

From our own experiments, it appears that cortisone is balanced and counterbalanced by other hormones, some of them appearing to have a direct antagonistic action by possibly competing with the very systems that are attacked by cortisone. Anterior pituitary growth hormone is capable of restoring the cortisone-suppressed fibroblast to normal. Desoxycorticosterone will also stimulate the inhibited fibroblast but, under its influence, the latter will lose its polarity and develop into a cell that is different from the normal fibroblast. This would indicate that desoxycorticosterone, or one of its metabolites, operates on a system different from the one on which cortisone or growth hormone operates. Considering the individual action of the various steroids upon granulation tissue, certain characteristic features of the hormones emerge that demonstrate that the effects can be well differentiated and differ from each other. Of the hormones studied, cortisone and growth hormone seem to be the only ones exhibiting these effects upon granulation tissue directly and immediately, which points to their great affinity to these structures. It appears possible, therefore, that cortisone operates in conjunction with other hormones, competing for a variety of factors regulating the formation of mesenchymal structures.

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EVALUATION OF STRUCTURAL AND CHEMICAL CHANGES IN CONNECTIVE TISSUE*

By Jerome Gross

Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts

Morphological studies on altered tissues must necessarily be based on adequate knowledge of the normal structure. Similarly, an understanding of the observed changes will be promoted by adequate studies of the range of reactivity of the tissue components individually and in the native complex under controlled conditions. To a large degree, the success of this approach to fundamental processes will be determined by the development of applicable methodology.

As long as the purpose of morphological characterization of a tissue component is description of changes on a histological level, such characterization is adequate. But, if it is agreed that disease processes involve chemical and colloidal changes, the present histological procedures are not as satisfactory. Thus far, the mechanism of staining in most histological methods is unknown and, moreover, there is little established empirical correlation between staining and the chemical and colloidal content or state of the tissue. As an example, in the case of collagen and reticulin, there is at present no knowledge of the nature of alterations that produce changes in staining reaction. In addition, in a histological section one observes alteration only in a total fiber of collagen and not in the collagen fibril itself. The fiber visible in the light microscope is actually a bundle of "submicroscopic" fibrils embedded in an amorphous matrix. Alterations could be due to changes in the fibril, in the matrix, or in both. Concerning the ground substance, there is accumulating a body of evidence from reliable sources to indicate that the periodic acid-Schiff reaction[1, 2, 3] and metachromasia[4] cannot be relied upon to demonstrate the presence of hyaluronic acid or chondroitin sulfate, the only well characterized mucopolysaccharides in connective tissue. Besides being nonspecific, these reactions give, at present, no idea of the quantity of material stained. They indicate neither the physical state of the stained matter nor their chemical relationships to other components. Controls with various hyaluronidases are difficult to interpret, since the purity and specificity of these enzymes are not completely established. Their substrates may be the links binding the stained material to the tissue and not necessarily the chromotrope itself.

The most-useful definitions leading to an understanding of physical and chemical alterations in the tissue components will be those that are intrinsic to the substances themselves. The defining characteristics are those of chemical composition, intramolecular order, and colloidal or macromolecular organization, since these properties give a substance its specificity and are directly involved in its alterations and reactions. The extracellular components of the connective tissue are well suited to such characterization by methods already available.[5-10]

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Definitions

The connective tissue in its broadest sense may be defined as a variable complex of extracellular substances and a variety of mesenchymal cells.[11] This definition should include vitreous humor, synovial fluid, and intercellular "cement." It is worth noting that there is no true extracellular space. Under normal conditions, the more or less gelatinous extracellular substance fills all space between cells and groups of cells.[12]

The commonest mesenchymal cell type is the fibroblast, which may be subdivided into a number of species distinguished by differences in function and metabolic characteristics.[13] These cells vary markedly in morphology with location, time and the chemical and physical properties of their milieu.[14, 15] Fibroblasts are most probably involved in synthesis and maintenance of the extracellular substances. In addition, there are the fixed and wandering macrophages, and the mast cell, whose function is still a mystery.[16, 17] To a great extent, characterization of the cellular components should be biological; *i.e.*, in terms of their arrangement in tissue, their interrelationships, and their functional role as important cogs in a larger machine. Their colloidal organization and biochemical behavior have become amenable to study, however.

Collagen is, at present, most adequately defined as a class of fibrous proteins having a characteristic wide-angle x-ray diffraction pattern and, in most known examples, an axial periodicity of about 640 Angstrom units.[18] Most of the collagens analyzed chemically are characterized by an unusually high content of glycine, hydroxylysine, and hydroxyproline, and an unusually low complement of aromatic amino and sulfur containing acids.[19, 20]

The nature of reticulin has been argued for decades, and the only universally accepted criteria for identification are its thin, branching, fibrous nature and its argyrophilia. Robb-Smith[21] claims that it is also nonbirefringent, in contrast to collagen. There has been much debate concerning its ability to gelatinize on boiling, its suceptibility to enzymatic digestion[21-25] and its amino acid composition.[26, 27] Available chemical analyses indicate an amino acid pattern not too different from that of mammalian collagen. The problem here is one of purity, since reticulin seems always to be associated with a certain amount of collagen and ground substance. Wide-angle x-ray diffraction studies of newborn rat skin corium[28] have demonstrated the fibrous component, which is nearly completely argyrophilic, to be a typical member of the collagen class, and electron microscopy has revealed the characteristic axial period of 640A.[5] These fibrils of reticulin appear to differ structurally from collagen in having smaller diameters.[5] Studies of reticulin from spleen[29, 30] and lymph nodes[30, 31] with the electron microscope have yielded similar results. Robb-Smith[21] and Little and Kramer[32] claim that reticulin from kidney, which is free of collagen, appears in the electron microscope as amorphous laminae in which are embedded thin fibrils with the collagen axial period.

Elastic fibers are present in most connective tissues and are always associated with collagen. They exhibit long-range rubberlike extensibility, have great tensile strength, are heat resistant, and are insoluble in most solvents. They are dissolved by an elastolytic enzyme in the pancreas[33, 33a] by concentrated formic acid,[34] and by boiling in $0.25N$ oxalic acid.[35] Elastin that has been thoroughly autoclaved to remove associated collagen has given only a diffuse uninterpretable x-ray diffraction pattern to date, hence cannot yet be included in the collagen class of proteins.[36] Stretched elastin fibers show positive axial birefringence, indicating the presence of axially oriented long chains,[37] but their electron-microscopic appearance is nondistinctive. Collagen fibrils are seen to pass into the substance of elastic fibers.[38] Elastin appears to be chemically distinguished by a large preponderance of nonpolar amino acids.[19, 39]

The ground substance is probably best defined as the extracellular, extrafibrillar amorphous matrix, which varies in content from tissue to tissue. In most loose and compact connective tissue, the ground substance is in a gel state. As synovial fluid it is liquid. This matrix consists of water, salts, nonelectrolytes, proteins, mucoproteins, mucopolysaccharides, and other substances, which are all probably in various states of interaction with each other. Constituents of the ground substance can be divided into two categories: one indigenous to the connective tissue, namely, the acid mucopolysaccharides, soluble collagen, and probably other proteins; and a second group consisting of transients derived from the blood and parenchymal cells. These latter may pass rapidly through the connective tissues or may be trapped or stored there. Much has been written on the composition of ground substance in several tissues, primarily with respect to carbohydrate content,[40, 40a] but studies on components other than carbohydrates have been few.[6, 7, 41] There has been little or no characterization of ground substance in parenchymal organs, where the passage of metabolites through extracellular tissue may be important to cellular function. Such studies will require the laborious but essential process of extraction, fractionation, purification, and chemical identification of constituents.

In the last several years, correlations have been made between the elevated serum α_1 acid glycoprotein in certain disease states with the breakdown of ground substance.[42-45] There is, however, as yet no good evidence for the presence of this acid glycoprotein, as characterized by Weimer *et al.*[46] and Schmid,[47, 47a] in connective tissue, although it may be present. Establishment of direct relationships between blood and tissue elements requires exacting chemical correlation. To date, the only carbohydrate components of connective tissue that have been isolated and adequately characterized are hyaluronic acid and chondroitin sulfate (a new carbohydrate-containing fraction has recently been isolated from cornea.)[6] It seems quite unlikely that the serum acid glycoprotein arises as a breakdown product of the mucopolysaccharides, because of the clear-cut chemical differences between them. The mucopolysaccharides are high-polymer carbohydrates containing *n*-acetyl hexosamine and glucuronic acid in equimolar quantities associated in long chains, of high molecular weight. The acid glycoprotein is a protein molecule strongly conjugated that does not contain glucuronic acid, the total molecular weight of which is about 60,000. The only known component they have in common is *n*-acetyl hexosamine.

Experimental

As one approach to the evaluation of changes in connective tissue, it has seemed worthwhile to establish the range of alteration certain purified components are capable of undergoing under the influence of various simple reagents. It is thus hoped to establish some base line for reactivity comparable to that for structure and composition.

Because the collagen fibril is distinguished in the electron microscope by a constant and characteristic "fingerprint," namely, the axial periodicity of about 640A. and detailed, distinctive intraperiod fine structure,[48, 49] the effect of a variety of agents in altering this structure appeared to be a good starting point.

Cowhide corium collagen was purified by a modified method of Bergmann and Stein[50] (10 days of repeated extraction of the diced corium with 10 per cent NaCl, followed by ten days of similar treatment with $M/15$ K_2HPO_4, then removal of salt by dialysis followed by lyophilization of the residue). Portions of this collagen were then freeze-sectioned to disperse the fibrils. The usual control preparations were made by allowing a drop of water suspension of fibrils to lie on the collodion film of the supporting grid for several minutes, followed by draining, staining with one per cent phosphotungstic acid and/or shadowing with chromium. Such fibrils as shown in FIGURE 1 are clean and relatively uniform in structure.

Aliquots of the sectioned collagen were then suspended in the following solutions for varying periods of time:

0.1N HCl	At room temperature
0.1N NaOH	
10 per cent guanidine HCl, pH 6.8	
0.1 per cent crystalline trypsin at pH 7.8	At 37 C.
0.1 per cent pepsin at pH 2.0	
at pH 4.0	
at pH 5.0	
0.05 per cent collagenase (MacLennan) at pH 7.6	

Aliquots were also incubated in the buffer solutions in the absence of enzymes.

At the end of several different periods of incubation ranging from 1 minute to 24 hours, suspensions in the acid, alkali, and guanidine were prepared for electron microscopy, as described above for the controls. The enzyme-treated suspensions were first sedimented at about 70,000 g. in the Spinco preparative centrifuge, the supernate poured off, and the residue of fibrils suspended in distilled water and prepared as above. The decanted supernatent fluids were also examined similarly.

Results

(1) Incubation of purified collagen fibrils in 0.1N HCl at room temperature causes rapid swelling. Within a few minutes, one may observe marked local distortions of the axial periodic structures in many of the fibrils with beginning evidence of fraying (FIGURE 2). Some fibrils appear unaffected. Frequently, fibrils that have been fragmented by excessive blending resemble these. Within

10 minutes, many fibrils are flattened and distorted all along their length (FIGURE 3). After several hours, there is a general destruction of the axial structure with marked swelling and flattening along the whole length of most of the fibrils (FIGURE 4). In some, the axial period is still evident, but there appears to be some shortening in the average spacing. In nearly all preparations, fibrils in all stages of disruption could be found, from completely normal to totally homogenized. With time, an increasing proportion of fibrils showed increasingly severe disorientation. Similar though much less pronounced effects were observed at pH 4 and 5.

(2) Incubation in $0.1N$ NaOH at room temperature for several hours resulted in marked fragmentation of fibrils, very frequently in a transverse direction and, in addition, total reduction of some fibrils to an amorphous mass (FIGURE 5). This is interesting in view of a widespread belief that dilute alkali does not appreciably attack collagen structure.

(3) Guanidine HCl (pH 6.5), which is a strong swelling agent, also causes local swellings and distortion of structure not too different from the early effect of dilute acid.

(4) Crystalline trypsin, as has been noted many times previously, has no observable effect on fibril structure.

(5) The effect of pepsin in the early stages of digestion is difficult to dissociate from the action of the acid milieu required for peptic activity. Even at pH 5, there is evidence of distortion of the axial structure in the control solution in the absence of the enzyme. After several hours, however, peptic activity is unmistakable; the collagen fibrils are nearly completely destroyed. At a somewhat earlier period, portions of the rare fibrils still identifiable show complete homogenization and large pebbly masses of unidentifiable material are predominant (FIGURE 6). After several more hours, even these disappear.

(6) The effect of collagenase prepared from clostridium hystolyticum* is extremely interesting.

The control preparations (FIGURE 1) uniformly show the severed ends of the collagen fibrils cut off relatively sharply by the microtome knife and exhibit little evidence of fraying and none of tapering.

The earliest evidence of collagenase activity on these fibrils is an obvious tapering and loss of substance at the fibril ends (FIGURE 7). After several hours, these fibrils show long stretches of markedly diminished diameter at the ends and often in the middle (FIGURE 8). Frequently, these thinned-out portions of fibrils are quite uniform over long distances. Quite characteristic of the action of this enzyme is the absence of any observable distortion in the axial periodicity.

In several preparations of intact human skin, rat-tail tendon, and purified cowhide that had been digested with collagenase, moderate numbers of long filaments resembling strings of beads with very regular periodicity of about 600-650A. appeared in all fields (FIGURE 9). These were usually associated with other tangled masses of short, thin, smooth filaments in addition to partially digested fibrils. Whether or not these structures are breakdown products of collagen or derive from some other residue of connective tissue is unknown. The fact that they are not present in large quantity after extensive digestion raises doubt as to their derivation from collagen fibrils. They are not present in preparations of collagenase incubated alone. Similar beaded structures have been seen in the vitreous humor,[51] in cartilage, and in healing wounds in scorbutic animals (unpublished). After digestion for 24 hours, there remains less than five per cent of solid material precipitable by prolonged centrifugation in the Spinco instrument at 144,000 g. Much of the dissolved material is non-dialyzable.

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* We are indebted to Dr. J. D. MacLennan for several samples of this enzyme.

Discussion

The simple experiments described in this paper represent an attempt to demonstrate the range of structural variation produced in a purified connective-tissue constituent by a few known agents. These agents are unphysiological as far as we know, but the alterations they produce in collagen illustrate some types of structural changes this protein is capable of undergoing. It should be noted that the reactivity of "naked" purified collagen fibrils may be different from that of fibrils embedded in ground substance in the tissue. Such differences in reactivity to acid between untouched fascia and trypsin-treated tissue (ground substance removed) has been observed in the dark field microscope by Day.[52]

These experiments demonstrate an important limitation (as well as the advantages), in pathological studies, of methods such as electron microscopy that rely for identification on the presence of more or less unique morphological "fingerprints," such as the axial repeating period in collagen. They are at a disadvantage in the examination of amorphous materials. Fibers whose structure is completely obliterated, such as some of those in FIGURE 4, could not be identified as collagen if found in a pathological lesion. The presence of the characteristic periodicity in part of the fibril, as seen in other fibrils in the same photograph, provides a label, however. It should also be noted that the periodicity of any individual fibril may range from 400 to 1000 Angstrom units (with a few rare ones outside these limits), although the great majority will lie between 550 and 700A.[29, 48] Therefore, to be rigorous in using the periodicity as identification, enough fibrils should be measured to give a characteristic distribution curve.

It is believed that a systematic stepwise investigation of controlled alterations, progressing from purified components to systems of increasing complexity, will not only lead to better understanding of the chemistry involved in physiologic and pathologic processes but may give more specific meaning to the observed histologic changes.

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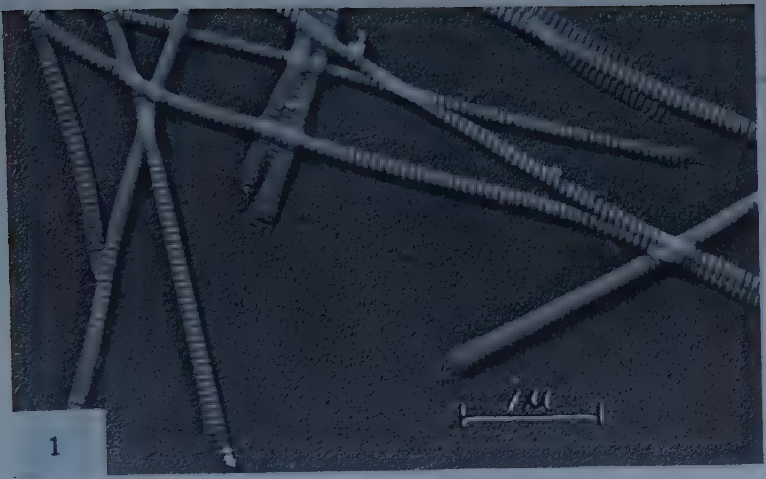

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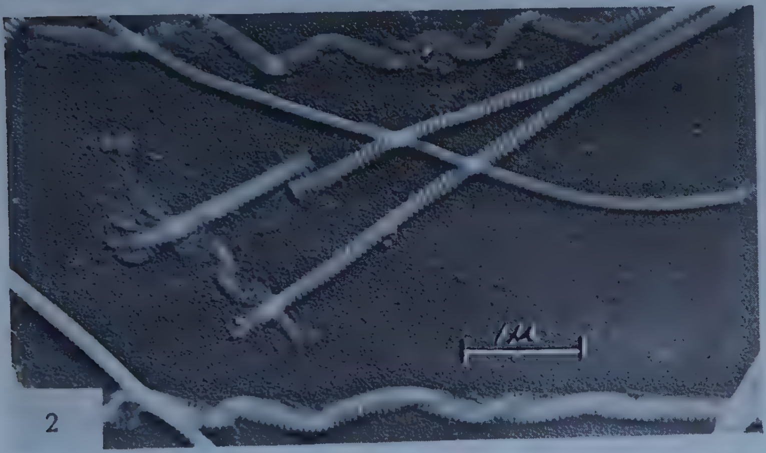

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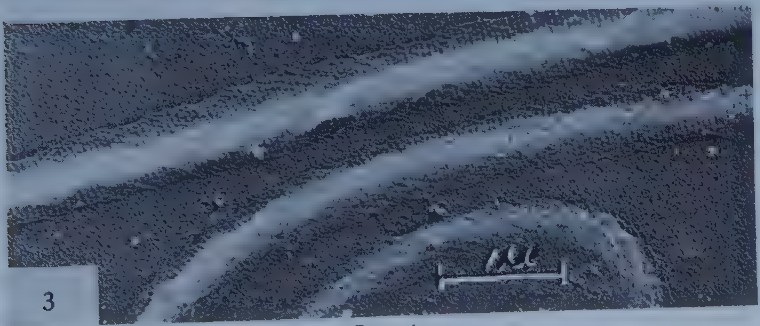

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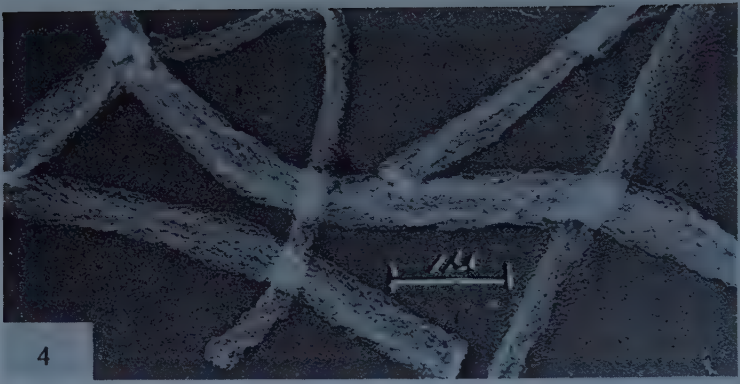
PLATE 1

FIGURE 1. Collagen fibrils from steerhide corium purified by the Bergmann procedure and shadowed with chromium. Note that the fibrils are uniform in diameter and the ends are cut off almost square. × 18,300.

FIGURE 2. Fibrils of same preparation which have been exposed to 0.1*N* HCl for 2 minutes. Note variability in degree of distortion. × 15,000.

FIGURE 3. Same preparation as in figure 2 but another field. Note flattening and some shortening of the period. × 15,200.



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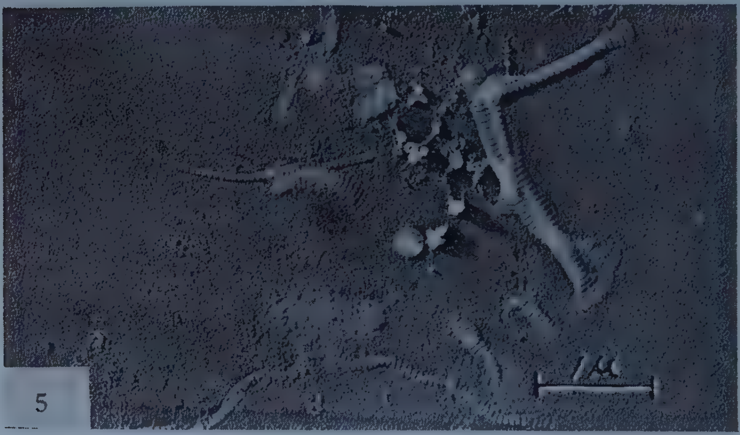

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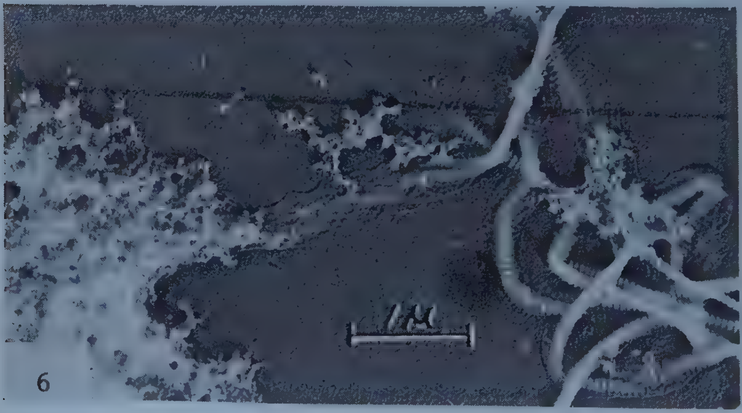

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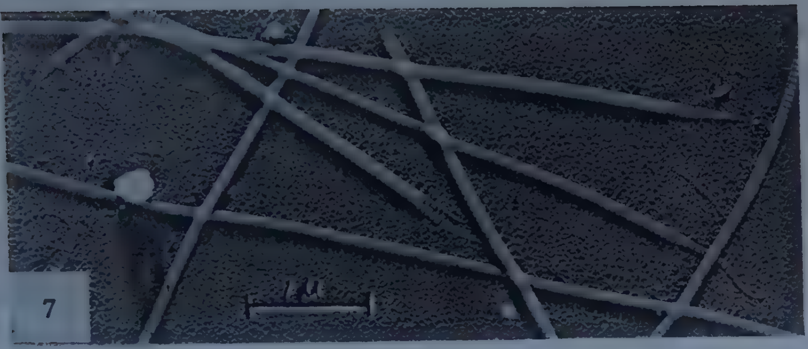
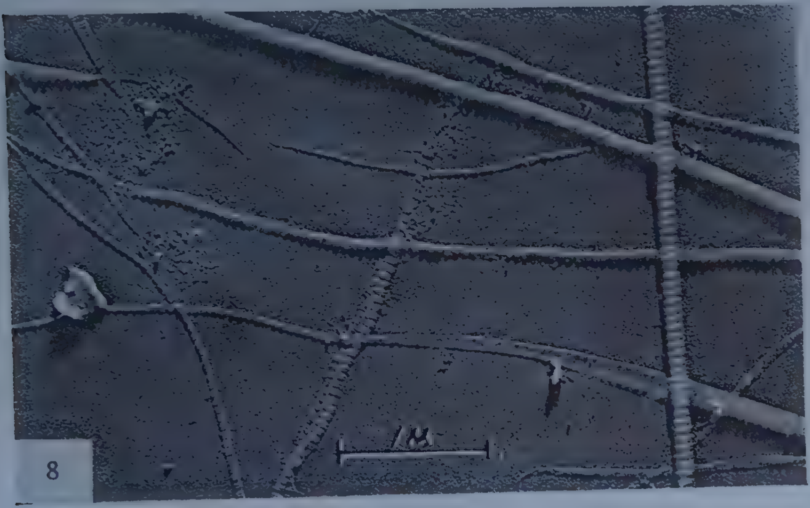
Plate 2

Figure 4. Collagen fibrils after 3 hours of immersion in 0.1N HCl. Note nearly complete loss of axial structure. Only two fibrils show remnants of the periodicity.

Figure 5. Collagen fibrils after 3 hour treatment with cold 0.1N NaOH. Note transverse fragmentation and flattening with loss of structure. × 14,700.

Figure 6. Effect of pepsin activity at pH 2.0 at room temperature on collagen fibrils. Note presence of essentially normal period in some fibrils, marked distortion in others, and complete reduction to a nondescript mass, × 15,300.





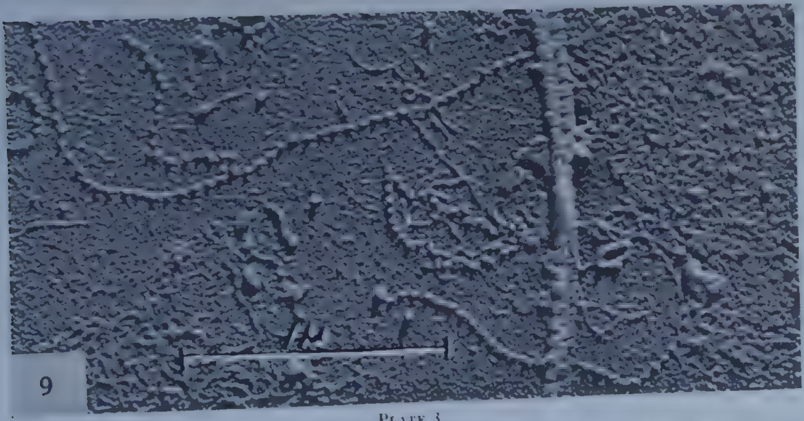


Plate 3

Figure 7. Collagen fibrils after 30 minutes of collagenase digestion. Note tapering and loss of substance at fibril tips with no loss of periodic structure. × 15,300.

Figure 8. After 4 hours of collagenase digestion there is marked tapering and thinning of portions of fibrils with no loss of axial structure. × 19,000.

Figure 9. Human skin corium attacked by collagenase. Note relatively intact fibrils, thin headed fibrils and masses of filaments. × 33,650.

MAST CELLS OF THE OMENTUM IN RELATION TO STATES OF ADRENOCORTICAL DEFICIENCY AND EXCESS*

By Burton L. Baker

Department of Anatomy, University of Michigan Medical School, Ann Arbor, Michigan

Little is known of the function of several components of loose fibroelastic connective tissue, the mast cells, in particular, having been resistant to experimental attack. Nevertheless, a discussion of them is appropriate to a conference on mechanisms of adrenocortical hormone action for several reasons. First, it is generally agreed that mast cells contain a mucopolysaccharide that, according to Lison[16] and Holmgren and Wilander,[14] stains metachromatically because of its esterification with sulfuric acid, the latter investigators concluding that this substance is heparin. Asboe-Hansen[3] reports that injection of corticotropin into man causes a marked reduction in the number of mast cells. Others claim that the coagulation time may be reduced[10] or prolonged, with an increase in the quantity of circulating heparin-like substances,[18] during treatment of human patients with corticotropin or cortisone. These effects require further study, however, because of the wide variation in the values obtained by the technical methods employed.[12]

Second, other workers believe that mast cells produce the mucopolysaccharide hyaluronic acid, which is an important constituent of the ground substance in connective tissue. Although not all histologists share this viewpoint, considerable inferential evidence may be marshalled to support it. Mast cells accumulate in the dermis bordering areas of epidermal hyperplasia resulting from applications of carcinogens to mouse skin. Cramer and Simpson[11] regard this occurrence as a possible protective action on the part of mast cells to supplement the hyaluronic acid of the dermal ground substance. The frequency of mast cells in myxedematous skin suggests to Asboe-Hansen[2] that they are the source of the increased amount of hyaluronic acid appearing in the dermis. He postulates that adrenocortical hormones may relieve inflammatory processes by reducing the capacity of mast cells to accelerate their production of hyaluronic acid.[3] Sylvén[19] holds that mast cells secrete the ground substance of granulation tissue. If this hypothesis is correct, mast cells merit consideration in experiments concerned with the effect of adrenocortical secretion on mesenchyme or fibroelastic connective tissue, because ground substance is one of the three constant components.

Third, mast cells are found with variable frequency in the connective tissue sheath of blood vessels, including small arteries (FIGURE 7), venules, and capillaries. If the hypothesis is correct that mast cells secrete ground substance, then it is conceivable that they may modify vascular permeability by altering the state of the mucopolysaccharides encompassing vascular endothelium. The irregularity of their occurrence would seem to militate against this possibility, however. Since so much evidence indicates that adrenocortical steroids

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* This investigation was supported (in part) by a research grant (A-131) from the National Institutes of Health and grants from Merck and Co. and the Upjohn Company.

aid in regulating the walls of blood vessels, determination of the changes that occur in mast cells in varying states of permeability appears to be worthwhile.

Finally, current information concerning the relationship of adrenocortical secretions to mast cells is conflicting and indefinite. According to Cavallero and Braccini[9] cortisone reduces the number of mast cells in sections of skin, muscle, and myocardium of the rat. Kelsall and Crabb[15] counted the number of mast cells per cross section of the thymus of hamsters treated with adrenocortical extract or cortisone. Their results are inconclusive because of the marked atrophy of the thymus which occurs under this treatment. The number of mast cells appears to be less than in *ad libitum*-fed controls but more than in inanition controls. Baker *et al.*[5] noted an increase in the concentration of mast cells in the thymic capsule of rats treated with corticotropin but regarded this outcome as secondary to the reduction in size of the thymus rather than resulting from a real increase in number. The need for more definite information on the relationship of the adrenal cortex to mast cells is emphasized by the dramatic regression of mastocytoma in one cortisone-treated dog recently reported by Bloom.[7]

This paper deals with the numerical and morphological variations which occur in mast cells when the concentration of circulating adrenocortical hormones is deficient or excessive.

Experimental Method. Precise study of mast cells is complicated by a dearth of knowledge as to their origin, a great normal variation in number, by the unusual fragility of the cell, and by the solubility in water of cytoplasmic constituents. To obviate at least the latter two complications, the observations to be reported were made on air-dried spreads of the omentum of rats that were stained by the May-Gruenwald-Giemsa procedure.

Mast cells are scattered throughout the omentum of the rat, including the fat that surrounds the larger blood vessels. They appear in greatest number in the milk spots and in the connective-tissue-surrounding capillaries, small arteries, and venules. Because of this positional relationship to capillaries, the number of mast cells is frequently great in the capillary network that borders the omental fat. Special attention will be directed to the effect of adrenocortical hormones on mast cells located alongside capillaries in avascular areas of the omentum, in milk spots, and at the edge of mesenteric fat. Examination of these different sites is necessary because mast cells migrate by amoeboid movement[17] and might change their location under the influence of hormones.

The mast cells bordering the omental capillaries (approximately 13–32 μ in diameter) were counted in each high-power view or 350 μ lengthwise section of the vessel an average of 32 sections being studied per animal. The mast cells of the avascular omental regions were ascertained by a differential count of the different types of cells that compose the omentum, over 1000 cells being counted per specimen.

The Effect of Adrenalectomy

If the normal morphology and number of mast cells is maintained by the level of circulating adrenocortical steroids, some change should be observed after adrenalectomy. Accordingly, adult male Sprague-Dawley rats were adrenalectomized and allowed to live 21–43 days before autopsy. The individual control animals of six adrenalectomized rats were pair-fed, while those of 11 adrenalectomized rats ate *ad libitum.* The latter 11 pairs of rats were given 4 cc. of 0.9 per cent NaCl intraperitoneally two hours before autopsy in connection with another experiment. This treatment with salt solution did not affect the mast cells. All adrenalectomized rats were maintained on a 1 per cent solution of NaCl as drinking fluid.

TABLE 1 shows that, following adrenalectomy, the paravascular mast cells increased slightly in number per unit length of capillary. Although this increase is small, it is obtained rather consistently. The number of mast cells was greater in the adrenalectomized member of five of six pairs whose food intake was the same as that of its control, and in 9 of 11 that ate *ad libitum.*

The small difference in the percentage of mast cells in avascular omental areas in adrenalectomized and control rats (1.3 and 1.7 per cent respectively) is of little significance because of the great variation in number and occurrence of fewer mast cells in only four of six operated rats.

TABLE 1

THE EFFECT OF ADRENALECTOMY ON THE MEAN NUMBER OF MAST CELLS

	No. rats	Days adx.	No./unit length of cap.	Per cent in avascular areas
Adrenalectomy	6	24–43	4.7 ± 0.8*	1.3 ± 0.5
Pair-fed controls	6	—	3.2 ± 1.2	1.7 ± 1.2
Adrenalectomy	11	7–41	7.4 ± 3.5	
Ad lib., controls	11	—	5.5 ± 2.7	

* Standard deviation.

The milk spots located in the fat surrounding the larger omental blood vessels were denser and more numerous per unit area in a majority of adrenalectomized rats and, frequently, the mast cells in them were more plentiful. Likewise, mast cells were more concentrated in the vascular network of the edge of omental fat (FIGURES 1 and 2). Evaluation of this change is difficult because omental fat was depleted consistently after adrenalectomy, with the possible result that mast cells were dispersed less widely. The blood vessels in omental fat were much wider in adrenalectomized than in control animals. This may have been due to a reduced tonicity of the vessel walls, the necessity for an increase in blood flow concomitant with a higher rate of metabolism in adipose tissue or the milk spots they supply, or a reduced external pressure resulting from resorption of depot fat, which would allow the vessels to assume a more normal caliber.

No variations in size or general morphology of mast cells occurred after adrenalectomy. Thus adrenalectomy appears to have little effect on omental mast cells of the rat, only a small increase in number bordering the capillaries being indicated.

The Effect of Hydrocortisone

Eight pairs of adult male Sprague-Dawley rats were used. One of each pair received 3 mg. of hydrocortisone (17-hydroxycorticosterone)* suspension daily for 10 days, the food intake of the control being limited to that of the hormone-treated rat. Four rats received the hormone suspended in 10 per cent ethanol and four received the hormone suspended in 0.9 per cent NaCl to which two small drops of Tween 80 were added per 20 cc. of fluid. Control animals were given the suspending medium.

No change occurred in the number of mast cells per unit length of blood vessel as a result of the action of hydrocortisone (TABLE 2). In seven of eight cases, hydrocortisone produced an increase in the percentage of mast cells in avascular areas with the difference of the means being small (1.65 per cent as compared with 1.17 for the controls). This does not indicate an absolute increase in number of mast cells because eosinophiles, lymphocytes and, to a slight degree, the fibroblasts, all dropped in percentage under treatment with hydrocortisone.

TABLE 2

THE EFFECT OF HYDROCORTISONE ON BODY WEIGHT AND MEAN NUMBER OF MAST CELLS IN THE OMENTUM

Treatment	No. rats	Mean per cent loss body weight	No. mast cells/unit length of cap.	Per cent mast cells in avascular areas
Hydrocortisone	8	19.5 ± 3.8*	5.2 ± 1.4	1.65 ± 0.53
Controls†	8	11.4 ± 2.2	5.1 ± 1.0	1.17 ± 0.55

* Standard deviation.
† Pair-fed.

Hydrocortisone had the reverse effect of adrenalectomy in the regions of omental fat, the amount of fat being increased and in some instances, the milk spots reduced in size. Mast cells were less concentrated at the edge of the omental fat, but this was probably due to greater dispersion resulting from the increased fat (FIGURES 3 and 4). Hydrocortisone, under these conditions, appears to have no effect on the number or morphology of omental mast cells.

The Effect of Cortisone on the Early Inflammatory Reaction Induced by Peptone

In order to study the participation of mast cells in the early vascular inflammatory reaction, adult male rats were adrenalectomized and, after 7 days, 4 cc. of a sterile 5 per cent peptone solution was injected intraperitoneally. Half of the rats received subcutaneously 5 mg. (1 cc.) doses of cortisone suspended in 10 per cent ethanol one hour prior to the peptone injection; simultaneously with it, and 3 hours after it; a total of 15 mg. being administered to each rat. Controls were given 10 per cent ethanol in place of the cortisone. The omenta were removed for study six hours after the peptone injection.

The intravenous administration of peptone to dogs causes a degranulation of mast cells in the liver and spleen but does not affect significantly those in the lungs.[21] The introduction of foreign protein (albumen) into the abdominal cavity of the rat has been reported to cause a disappearance of mast cells from the omentum.[20] In our experiments, we desired to study the response of mast cells to intra-abdominally administered foreign protein, particularly with respect to concurrent vascular changes and the presence of varying levels of circulating adrenocortical steroids.

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* We wish to express our appreciation to Dr. Elmer Alpert of Merck and Co. Inc., for the hydrocortisone and cortisone he supplied for these studies.

Within six hours after the introduction of 5 per cent peptone into the abdominal cavity of the rat, some mast cells apparently release part of their cytoplasm, liberating the contained granules into the surrounding connective-tissue matrix. In the immediate vicinity of these mast cells, stellate and round cells appear, which contain sparse, blue granules. One might assume that they represent newly forming mast cells or phagocytic cells that have engulfed the liberated mast cell granules. The latter explanation seems to be more tenable. This response typifies the mast cells located in avascular areas of the omentum but more strikingly those along blood vessels. In the latter location, phagocytosis of mast cell granules by stellate clasmatocytes is unusually common. Cortisone failed to protect mast cells from loss of granules and also did not modify significantly the uptake of release granules by paravascular clasmatocytes (FIGURES 5 and 6). Nevertheless, cortisone exerted a profound effect in reducing the amount of congestion of the small blood vessels and the magnitude of polymorphonuclear leucocytic migration out of the capillaries. Thus, with this experimental procedure, there was a clear-cut effect by cortisone on the capillary wall and the capacity of leucocytes to move through it, without any modification in the response of mast cells to peptone being detected. Therefore, it appears that, in this early type of inflammation, cortisone modifies the capillary wall without the apparent participation of mast cells. This conclusion is suggested further by the irregular occurrence of mast cells along these vessels.

Correlative Comments

These observations suggest that adrenalectomy and administration of hydrocortisone do not have a marked effect on the mast cells of the rat omentum, although a small increase in number of those located along blood vessels was indicated after adrenalectomy. Some reconciliation of these observations with those of Cavallero and Braccini[9] should be sought, since these workers found that cortisone acetate reduces the number of mast cells in skin, myocardium, and muscle of the rat. In their experiments, no account was taken of the difference in food intake between treated and control animals. At present, it is not known how much this factor could have modified their results.

Second, higher doses of cortisone were administered for shorter periods of time (10 mg. for three days) and lower doses for longer periods (up to 70 days) than were employed in our experiments. Also, Bloom[7] employed the rather high dose of 100 mg. of cortisone per day in order to induce regression of canine mastocytoma.

Third, there is no proof that mast cells in all parts of the body perform the same function. Indeed, some evidence indicates that the mast cells of various locations differ in their reaction to experimental procedures. The omental mast cells may be more resistant to adrenocortical hormones than those in myocardium or skin. Local treatment of rat skin with adrenocortical hormones (extract or cortisone) causes a reduction in size, granulation, and number of mast cells in the dermal reticular layer in the area of application but they remain unchanged outside of the treated area. The mast cells located just beneath the epidermis are affected most severely. This change is obvious upon cursory examination of sections of skin. The magnitude of reduction is shown by TABLE 3, which gives the number of mast cells observed in the dermis of the right and left sides of the neck of rats, the right side having been treated daily with 100 μg. of cortisone (0.1 cc.) in 25 per cent alcohol for 133 days, the controls receiving equal volumes of the solvent. The number of mast cells was determined in strips 350 μ wide extending from the epidermis into the inner border of the portion of the dermis that contains elastic fibers. Since only collagenous fibers are reduced by the hormone, the extent of elastic fibers outlined a comparable amount of dermis in both treated and nontreated skins. The specimens were stained with Gomori's aldehyde–fuchsin procedure which reveals both mast cells and elastic fibers. Cortisone caused a 64.5 per cent reduction in number whereas alcohol reduced them only 4 per cent.

TABLE 3

THE EFFECT OF LOCAL TREATMENT WITH CORTISONE ON THE NUMBER OF MAST CELLS IN THE DERMIS

Treatment	Mean no./unit area		Mean % reduction treated side
	Treated side	Nontreated side	
Cortisone	1.4 ± 1.0*	3.8 ± 1.4	64.5
25% Alcohol	3.3 ± 1.5	3.6 ± 1.4	4.0

* Standard deviation.

It must be remembered that prolonged local treatment of the skin with adrenocortical steroids will induce far more profound structural modification of the dermis than can be elicited by systemic administration of either cortisone or corticotropin. One is led to the supposition that, when mast cells are reduced by cortisone, the effect is a manifestation of exaggerated physiological action due to great overdosage.

The hypothesis that mast cells secrete a component of the ground substance should be weighed against the possibility that fibroblasts perform this function. Several observations of the behavior of fibroblasts in states of local or systemic hyper-adrenocorticalism indicate that their activity is suppressed. They remain small and fail to proliferate at a normal rate in the formation of granulation tissue.[1, 6] Concurrently, the local accelerated spreading action of hyaluronidase, which typifies the skin of these rats, could result from retarded production of hyaluronic acid by the fibroblasts.[13] When the number of mast cells is reduced in rat skin treated locally with adrenal steroid hormones, the fibroblasts are affected similarly.

It is hoped that the negative viewpoint expressed in this presentation with respect to the effect of adrenal hormones on mast cells will not discourage other investigators from studying them further. The experimental methods we and others have employed are not sufficiently varied to permit a final conclusion concerning the participation of mast cells in the induction of adrenocortical hormone effects. Nevertheless, the current enthusiasm for mast cells as the site of formation of ground substance should not cause us to ignore the other leading candidate for the honor—the fibroblast.

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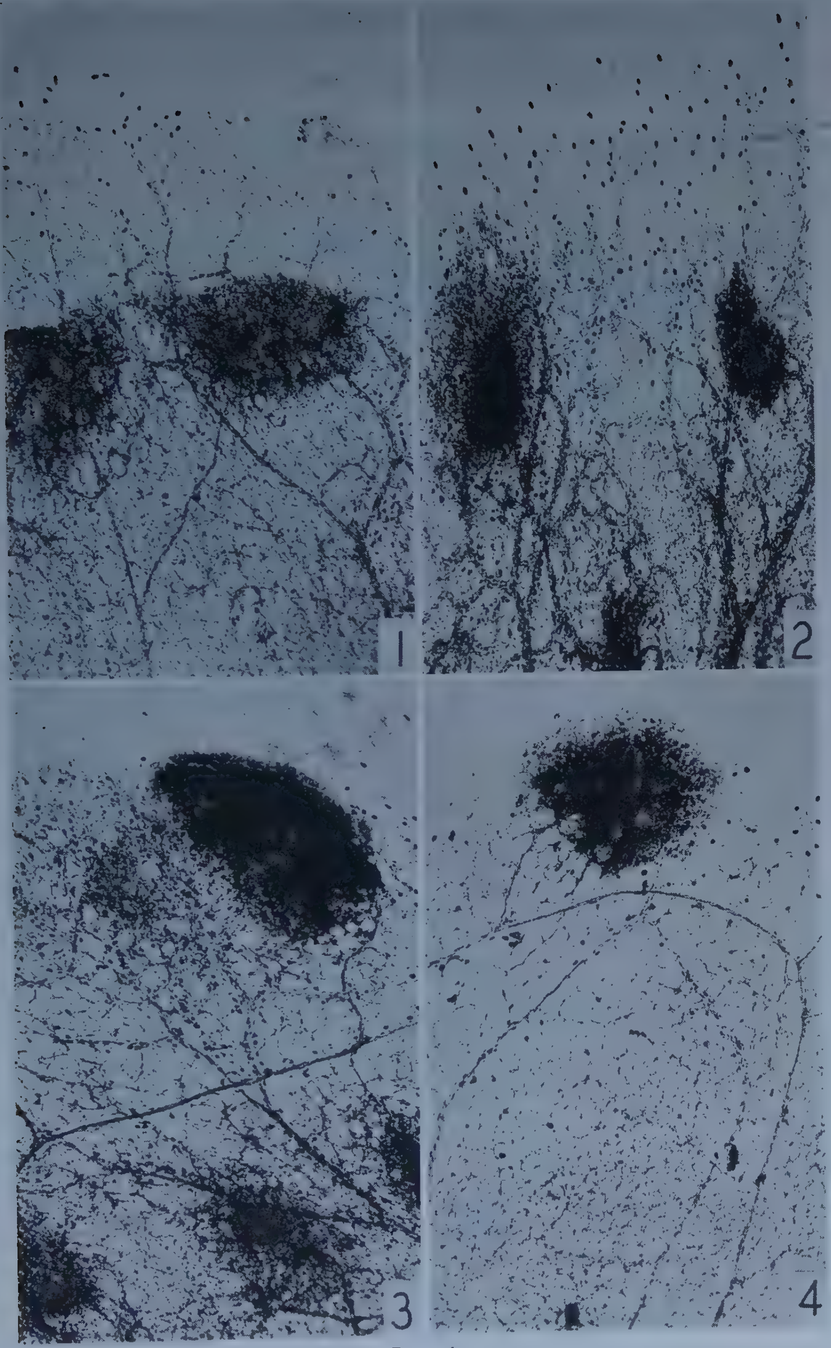


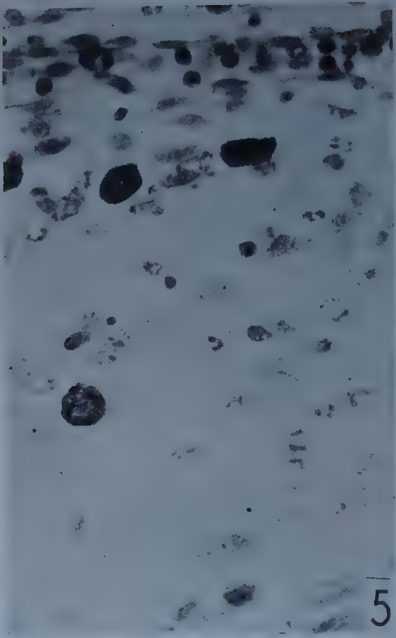
PLATE 1

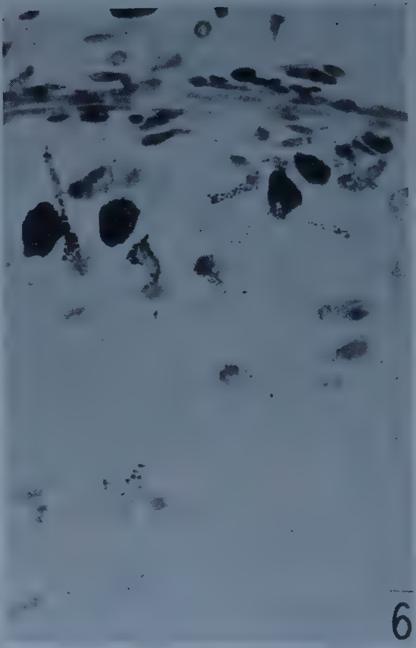
FIGURE 1. Pair-fed control for specimen illustrated in FIGURE 2. Periphery of omental fat showing scattered mast cells in capillary network above, and in milk spots. Fat cells (vacuolated) are large and blood vessels narrow.

FIGURE 2. Adrenalectomized for 43 days. Mast cells are more concentrated in capillary network and in the denser milk spots. Fat is reduced and blood vessels are wider.

FIGURE 3. Pair-fed control for specimen illustrated in FIGURE 4. Numerous mast cells are present at the edge of the fat.

FIGURE 4. Three mg. of hydrocortisone daily for 10 days. Mast cells are less concentrated, milk spots less frequent. The greater deposition of fat is accompanied by narrower blood vessels.





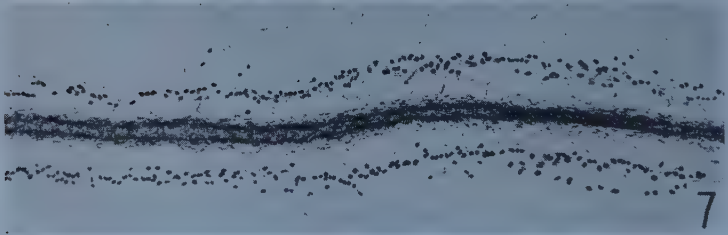


PLATE 2

FIGURE 5. Adrenalectomy, 5 per cent peptone intra-peritoneally, control for FIGURE 6. A slightly congested capillary is at the top. Paravascular mast cells below it have given up some granules to intervening clasmatocytes. Polymorphonuclear leucocytes are frequent.

FIGURE 6. Treatment as for FIGURE 5 except cortisone given subcutaneously. The capillary is less congested and fewer leucocytes are present in the connective tissue. Paravascular clasmatocytes have taken up mast-cell granules.

FIGURE 7. Accumulation of mast cells around a small artery, 41 days after adrenalectomy.

EVIDENCE FOR THE DIRECT EFFECT OF STEROIDS ON THE GROUND SUBSTANCE

By Joseph Seifter, William E. Ehrich, David H. Baeder, Arthur J. Butt, and Ernst A. Hauser

Wyeth Institute of Applied Biochemistry, Graduate School of Medicine, University of Pennsylvania, Philadelphia General Hospital, Philadelphia, Pennsylvania; Butt Medical Foundation. Pensacola, Florida; Massachusetts Institute of Technology, Cambridge, Massachusetts

In 1947, we investigated the possibility that an alarm reaction might be beneficial in alleviating some of the pathological lesions that occur during the resistance stage to another stressing agent, and that these beneficial changes might involve alterations in the permeability of the ground substance. This consideration was prompted by observations made in our laboratories, where investigations on the role of the alarm reaction in simple toxicity,[1] the pharmacology of hyaluronidase,[2] and experimental serum disease were in progress.[3] Accordingly, we studied the effects of toxic doses of salicylates on the course of experimental serum disease, and concluded that massive salicylate therapy has little effect on rheumatic fever after the disease has been established, *i.e.*, after antibodies have been formed, but that it could be quite effective as a prophylactic measure if given early enough to depress antibody formation. We also concluded that a major effect was due to the stressor or toxic action. Colchicine, a more potent stressor, was found to be a more effective agent in the treatment of experimental serum disease. It appeared, therefore, that the therapeutic effects of salicylates and colchicine involved stimulation of the adrenal cortex.[4]

During the course of experimental serum disease, the ground substance of the hematoparenchymal barrier is readily permeated by antigen and antibodies. This so-called barrier is readily attacked by testicular hyaluronidase, which depolymerizes hyaluronic acid and chondroitin sulfate. Purified hyaluronidase has no demonstrable effects on permeability of blood vessels as measured by ordinary pharmacologic techniques, excepting in the rat.[5] In the latter species, however, the effect may not be specific, because high-molecular-weight substances, such as Dextran, are capable of altering capillary permeability.[6] This discussion will consider only the *in vivo* method for quantitatively measuring permeability across the synovial membrane in the talocrural articulation of the rabbit, which represents a special ground substance membrane. Other species, including the human, and other joints in the rabbit have not yielded consistent data. The original studies were limited to the effects of hyaluronidase and stressing agents on permeability. When the therapeutic effects of cortisone and ACTH were disclosed, we directed our studies to these substances and a variety of steroids.

TABLE 1 presents a statistical analysis of the reliability of the method. In standardization experiments in which no drug was administered, the standard deviation of the rate of flow was ±6.01 per cent in 1658 rabbits. The rabbits were standardized in 16 groups. If one rabbit is taken at random from each group, the standard deviation of the rate of flow is ±8.03 per cent. These results indicate that an effect on permeability can be established by using two to three rabbits. Determination of the effects of ACTH, DCA, cortisone acetate, and 21-acetoxypregnenolone on an excess number of rabbits was of the same order of accurary. In studies involving all compounds tested, more than 5000 injections into the talocrural articulation have been made. These studies establish that hyaluronidase and desoxycorticosterone markedly increase permeability across the synovial membrane, and that ACTH and cortisone have marked antipermeability action.

Cortisone and ACTH decreased permeability 20-fold over normal; DCA and hyaluronidase increased it 2-fold. The antipermeability effects of cortisone can be partially antagonized by the administration of a 5-fold excess of DCA or a 10,000-fold excess of hyaluronidase. From these data, a hypothesis can be proposed that the common site of action of cortisone, DCA, and hyaluronidase in the ground substance is the mucopolysaccharides which are acted on by the enzyme. Although we have attributed an antihyaluronidase action to cortisone, we have been unable to demonstrate this effect in the *in vitro* assay for hyaluronidase. The antihyaluronidase action is probably an alteration of the mucopolysaccharides by cortisone, so that these became resistant to attack by the enzyme. DCA also renders the ground substance more resistant to attack by the enzyme, so that it is not possible to augment DCA enhanced permeability by administration of the usual dose of hyaluronidase.

TABLE 1

STATISTICAL ANALYSIS OF STUDIES IN SYNOVIAL MEMBRANE PERMEABILITY IN RABBITS

Treatment	No. of rabbits	Dose	Route	Mean rate of flow*	Standard deviation	
					± μg. PSP/min.	± %
Standardization	1658 (16 groups)	—	—	18.135	1.08	6.01
Standardization	16 (one from each group)	—	—	17.818	1.432	8.03
ACTH	14	0.25 mg./kg.	IV	0.719	0.050	7.03
DCA	10	5 mg.	IM	44.985	0.587	0.13
Cortisone acetate	10	5 mg.	IM	1.286	0.014	1.10
21-Acetoxypregnenolone	12	5 mg.	IM	1.052	0.09	0.71

*μg. PSP/min.

In the course of screening numerous steroids we encountered 12 that had marked antipermeability, anti-DCA, and antihyaluronidase properties in intact rabbits.[7] It became necessary to determine whether the effect of these steroids was mediated through an effect on the adrenals. Adrenalectomy results in depression of the normal rate and amount of PSP cleared, particularly during the first hour. It abolishes completely the marked antipermeability effect of ACTH.[8, 9] This test is a useful indicator of successful adrenalectomy in rabbits. The marked antipermeability action of 21-acetoxypregnenolone and allopregnane-21-ol-3,20-dione 21-acetate in normal rabbits could not be elicited after adrenalectomy, whereas the action of cortisone was not affected by the operation. Since the activity of the first two steroids was not altered by hypophysectomy in rabbits with intact adrenals, it is assumed that the effect of these two compounds is mediated through the adrenals.

It has been suggested that the therapeutic benefits of cortisone may be due to this anti-DCA and antihyaluronidase actions. Cortisone, 21-acetoxypregnenolone, and the allopregnane compound antagonize the increased permeability resulting from the administration of DCA or hyaluronidase in intact rabbits.[7] Although adrenalectomy abolishes the antipermeability effects of 21-acetoxypregnenolone and the allopregnane compound, it does not abolish their anti-DCA and antihyaluronidase actions. The therapeutic and antipermeability properties of cortisone are probably not dependent exclusively on anti-DCA or antihyaluronidase actions. This is borne out in the failure of 21-acetoxypregnenolone to alter the course of experimental serum disease while cortisone protected the heart and blood vessels.[10]

Evidence for the direct effect of steroids on the mucopolysaccharides is also suggested by the demonstration that subcutaneous injection of hyaluronidase results in the clearing of turbid urines in stone formers and in the retardation of encrustations in indwelling catheters.[11] This observation has been confirmed by several investigators.[12]

Experimental studies in rabbits revealed a similar effect on the turbidity of the urine. Normal rabbit urine contains suspended material which, on centrifugation, results in a sediment of 1 ml. per 10 ml. of sample obtained by catheterization. In rabbits treated with hyaluronidase the sediment is reduced to 0.5 ml. per 10 ml. of urine. The effect of adrenalectomy approximates that of hyaluronidase treatment. In adrenalectomized rabbits receiving hyaluronidase, there is little or no sediment present.

Microscopic examination reveals that normal urine contains particulate matter dispersed by means of coating with a protective colloid. The urine of stone formers is characterized by the presence of crystals without protective colloid. Injection of hyaluronidase results in a coating of the crystals by a protective colloid. These findings have been described in detail by Butt and Hauser.[13]

The clarification of the turbid urine following injection of hyaluronidase is accompanied by a dispersion phenomenon that cannot be induced by the addition of hyaluronidase to the urine. Hyaluronate added to the urine results in a powerful peptizing and dispersing action.[14] Preliminary studies indicate that the subcutaneous injection of hyaluronidase in humans results in a significant elevation in the urinary levels of glucuronides and total reducing substances detectable by the Folin-Wu method, but not by the Benedict quantitative method.[15] There is no significant effect on calcium, phosphorus, or sulphur excretion. These findings suggest that the injection of hyaluronidase results in urinary excretion of hyaluronic acid.

The hypothesis is advanced that the injection of hyaluronidase results in the release of depolymerized hyaluronate from the site. The hyaluronate is excreted into the urine where it acts as a protective colloid. Further excretion of hyaluronate occurs during the period of restoration at the site of injection, although it may be purely coincidental that the effects of hyaluronidase injection on the urine parallel the period of reconstitution of the barrier.

Approximately 50 per cent of normal human males subjected to high-speed centrifugation show marked decrease of the protective colloids in the urine.[16]

The urine becomes turbid and contains an abundance of crystal particles within 15 minutes after exposure, and is restored to normal in approximately two hours. The sedimentation is not due to a change in pH or to marked phosphoturia. Similar urines have been obtained by the administration of ACTH and, sometimes, by the administration of cortisone. Microscopic examination of these urines likewise reveals the presence of penniform crystals that are indicative of a decreased protective colloid action. In preliminary studies it has been observed that cortisone decreases the excretion of the total reducing substances, excluding those detected by the Benedict method, and the total glucuronide, without markedly affecting the total phosphorus or sulphur. In one case of rheumatic fever, 300 mg. of cortisone on two successive days reduced the total reducing substances from an average of 2.05 g. per 24 hours to 0.967 g. in the first 24 hours, and 0.393 g. in the second 24 hours; and the total glucuronide from an average of 1.62 g. to 0.082 g. in the first 24 hours, and 0.034 g. in the second 24 hours. The decreased excretion of total reducing substances and glucuronide coincided with clinical improvement.

Summary

(1) Adrenal steroids have a direct effect on mucopolysaccharides that are depolymerized by hyaluronidase.

(2) Cortisone depresses or inhibits, while DCA augments the actions of these mucopolysaccharides.

(3) The antihyaluronidase action of adrenal steroids probably does not involve inactivation of the enzyme, but an alteration of the substrate.

(4) Of the adrenal steroidlike compounds tested, only cortisone and DCA exert their actions in the absence of the adrenals.

(5) The antipermeability effect of such compounds is abolished by adrenalectomy, the anti-DCA and antihyaluronidase actions are not.

(6) Therapeutic effects of cortisone in collagen disease do not depend entirely on antihyaluronidase or anti-DCA actions.

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Discussion of the Paper

Doctor Valy Menkin (*Temple University School of Medicine*, Philadelphia, Pa.):*

Cortisone acetate (Merck) (2.5 mg. in 10 cc. of sea water) tends to suppress the incidence of normal cleavage in fertilized *Arbacia punctulata* ova. This is not referable to the vehicle in which the steroid is suspended. The vehicle does not alter the cleavage pattern. Filtering the suspension of cortisone acetate yields a cortisone residue capable of suppressing the incidence of normal cleavage, whereas the filtrate, which is essentially the suspending vehicle, has no such inhibitory effect. The sperms are likewise inactivated by cortisone acetate (2.5 mg. in 10 cc. of sea water). Compound F suspended in propylene glycol likewise reduces the incidence of normal cleavage in fertilized *Arbacia* ova.

Desoxycorticosterone acetate and the glucoside tend to have the same inhibitory effect on the cleavage of ova.

ACTH (corticotropin) (Armour and Organon products) directly affects the cleavage pattern of *Arbacia* ova. There is also a greater incidence of suppression. This indicates that this pituitary hormone can likewise have a direct toxic effect on the ova without the mediation of the adrenal cortex.

In contrast, a protein anabolic steroid, testosterone propionate (2.5 mg. conc.) (Ciba), has no effect on the incidence of normal cleavage in *Arbacia* ova. Somatrofin (STH) (a Horner product), a growth hormone from the anterior pituitary, has likewise no effect on the cleavage pattern.

The suppressing action of ACTH is not a protein effect and is not due to any differential solubility of the hormone.

The specific results suggest that the direct suppression of cellular activity, as measured by cell division, of cortisone and ACTH throws light on the mechanism of suppression of these hormones in inflammation, as reported in 1940 and 1942 by the writer.

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* See also paper by Leonard and Rutstein on steroid compounds and single cells, pages 704–714 of this monograph.

THE EFFECTS OF ADRENAL HORMONES ON CONNECTIVE TISSUES*

By Albert Dorfman

Department of Pediatrics, University of Chicago and LaRabida Jackson Park Sanitarium

The striking effects of certain adrenal steroids on "diseases of connective tissue" has focused attention on the possibility that these hormones might exert a direct influence on this tissue. Several contributions to this monograph have outlined specific experimental observations supporting this thesis. The reported studies are now both numerous and diverse in nature. Attempts at interpreting these data require knowledge not only of the general biological effects of the adrenal hormones, but also a general conception of connective tissue.

It is evident that certain difficulties are presented by delimiting the problem of connective tissue, particularly since overlap with other closely related mesenchymal derivatives cannot be avoided. Thus, it is difficult to draw lines between the various sections of this symposium and isolate problems of connective tissue from those of bloods elements and vascular phenomena. Despite adherence to a limited view of connective tissue, generalizations regarding its behavior are difficult because of its variable characteristics, (*e.g.*, contrast the differences between bone and synovial fluid).

Although connective tissue is, in general, distinguished by a relatively large extracellular component, few investigators doubt the probable importance of the cellular elements in determining its behavior. There seems little question that the collagen fiber results from the metabolic activity of the fibroblast, although the evidence is less certain that this cell type is responsible for the production of mucopolysaccharides of the ground substance. Asboe-Hansen[1] has recently suggested that mast cells are responsible for the formation of mucopolysaccharides, although no definitive evidence is available that this is the case. It is unnecessary to consider in detail the many reports that indicate change in fibroblast activity under the influence of adrenal hormones. The early studies by Baker and Ingle[2] indicated that the administration of adenocorticotropin results in inhibition of fibroblast proliferation. More recent studies by Ragan and co-workers,[3, 4] which have been amply confirmed by others,[5, 6, 7] demonstrate that, under certain conditions, the administration of ACTH and cortisone results in repression of fibroblast proliferation and differentiation. Baker and co-workers[8, 9] showed similar effects following the local administration of adrenal extract. The studies of Taubenhaus[10, 11] and Dougherty[12, 13] on the inflammatory reaction further suggests inhibition of fibroblast proliferation.

Although it is quite clear that changes are observed, the basic meaning in terms of the essential locus of corticoid activity is not as evident. Such effects may result from a wide variety of influences. Inhibition of a fundamental

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biochemical reaction necessary for the multiplication and differentiation of fibroblasts may well be responsible. Such a reaction need not have any specificity for this series of cells, but may be common to many types of cells. The striking effect on connective tissue cells may result only from the experimental conditions, which focus attention on the proliferation and differentiations of such cells. Green[14] has suggested that the inhibition of mitosis is responsible for the cellular effects of cortisone.

Although it is satisfying to our present concepts to consider a direct inhibition or stimulation of enzymatic processes, studies on the mechanism of action on various hormones have unearthed a paucity of such examples. It would seem wise to consider the possibility that these influences on cellular physiology may derive from changes that are brought about in other ways. Thus, changes in enzymatic activity might result from disturbances in physical chemical state or composition of the milieu either at a cellular or at a tissue level. The known effect of adrenal hormones on salt and water metabolism must be considered in this regard.

Consideration of the effects of cortisone on extracellular elements is limited by the lack of knowledge concerning origin and metabolism of these substances. The manner in which the collagen molecule is fabricated is not understood. Equally important is our lack of knowledge concerning the course of events leading from the production of the simplest collagen molecule to the formation of the fibers at various levels of organization. It is obvious that interference with the final production of collagen may result from interference at any point along this chain of events. The recent studies of Orekhovitch and coworkers[15, 16] suggesting the existence of a soluble collagen precursor ("procollagen") obviously adds to the potential sites of action. The early observations of Nageotte,[17] together with the more recent studies of Gross, Highberger, and Schmidt,[18] and Porter and Vanamee[19] indicating that collagen fibrils may be formed *in vitro* as a result of changes in ionic strength or the addition of various substances such as blood mucoproteins obviously open up new avenues of approach with regard to possible loci of hormonal influences.

In recent years, great emphasis has been placed on the role of the ground substance of connective tissue in various physiological and pathological states. The histochemical studies of Gersh and Catchpole,[20] and of Altschuler and Angevine[21] have emphasized changes in ground substance although the methods employed are probably not adequate to draw definitive conclusions regarding the chemical nature of these changes.

Before considering the various contributions regarding the effects of adrenal hormones on ground substance, it would seem necessary to consider the meaning of the term "ground substance." This term is variously employed in the literature as referring at one extreme to any type of intracellular-fluid system, or at another to a specific chemical substance. With this wide range of ideas concerning ground substance, it is obviously difficult to interpret the meaning of changes observed or ascribed to ground substance. It would seem worthwhile to more specifically delineate our ideas. It is proposed that the term ground substance be used to denote the amorphous (to the limits of resolution of the electron microscope) continuum existing between the cells and vascular system. With such an inclusive definition, it becomes obvious that this extracellular compartment must be composed of a variety of components of diverse origins, and that the composition of this compartment will vary from time to time and place to place. It would be equally wrong to consider ground substance, either as a mysterious unknown quantity, or as a uniform chemical substance. On the basis of present knowledge, it becomes possible to consider the composition and factors that influence the nature of this portion of the body as follows:

(1) Substances derived from the blood stream, having a remote origin.
  (a) H_2O
  (b) Inorganic ions
  (c) Glucose, *etc.*
  (d) Blood proteins
  (e) Other unknown components
(2) Metabolic products of parenchymal cells
(3) Metabolic products of connective tissue
  (a) Fibrous structure (imbedded in ground substance)
    (1) Collagen
    (2) Elastin
    (3) Reticulin (?)
  (b) Mucopolysaccharides
    (1) Chondroitin sulfuric acid
    (2) Hyaluronic acid
    (3) Mucopolysaccharide protein complexes
    (4) Others (?)
  (c) Soluble proteins
    (1) Procollagen
    (2) Others (?)

Consideration of this outline of the ground substance reveals immediately that this represents only a generalization of all of the substances present in various areas. The individual composition of the ground substance differs at different sites and under a variety of both physiological and pathological conditions. The number of factors influencing the nature and composition of this mixture is obviously large. These factors include both those arising as a consequence of the materials deriving from the circulation and those arising as a consequence of the activity of the parenchymal cells. In addition to these influences, which may be considered as extrinsic to the connective tissue, the components peculiar to the connective tissue probably have a real and important function. Although evidence is as yet fragmentary, it seems reasonable to suppose that the existence of the mucopolysaccharides and proteins in the ground substance presupposes consideration of the extracellular fluid as merely an ultrafiltrate of blood plasma. There can be little doubt that the mucopolysaccharides are capable of binding inorganic ions, proteins and water, and as such may act as important regulators of the nature of the milieu in which parenchymal cells function. As a consequence of these properties, they may actually serve to regulate transport between circulation and parenchymal cells. Undoubtedly, a reciprocal relationship exists in that there is evidence that the molecular state of the polysaccharide (*e.g.*, electrical charge, molecular size, and configuration) is profoundly influenced by the ionic environment.[22, 23]

These concepts are of importance in attempting to interpret various studies with respect to the effect of adrenal cortical hormones on connective tissue. If one considers the problem of permeability of connective tissue, it is evident that various influences may change this property, and that influences having the same net result may be quite different in their mechanisms. Although there is no satisfactory direct evidence that permeability of connective tissue is directly related to the concentration and molecular state of the polysaccharides (or their protein complexes), it seems reasonable to assume that these are related properties. If we consider the factors that may affect the contribution of such substance to the permeability of connective tissue, it becomes apparent that changes in permeability may be dependent on various factors as outlined. Thus, if the fibroblasts (or other connective-tissue cells) increase the secretion of mucopolysaccharides, so that the concentration of this component rises in the ground substance, a change in the spreading phenomenon may occur. That similar results may be achieved as a result of far different factors is illustrated by the findings that the electrical charge and molecular configuration of chondroitin sulfuric acid is markedly altered by changes in ionic environment.[22] Thus, a primary process affecting inorganic-ion concentration may change permeability in the same way as the one that would result from changes in quantity of polysaccharide formed. It becomes quite obvious that apparently specific effects may have widely different origins and give no real clue as to real locus of action.

One of the most difficult problems that has to be dealt with in terms of mechanism of action of hormones is the problem of specificity of end-organ response. The ability of different types of connective tissue to become highly specialized in various locations indicates that this type of specificity applies to the behavior of connective tissue as well. A mechanism of adrenal hormone activity has been suggested by Layton.[24] This investigator found evidence of inhibition of synthesis of chondroitin sulfuric acid in embryonic and wound tissues maintained *in vitro*. These studies were based on the incorporation of radioactive sulfate as a measure of chondroitin sulfuric acid synthesis. Although this work is opened to the criticism that the chondroitin sulfuric acid was not isolated and identified, it is quite apparent that marked changes in the incorporation of radioactive sulfate were observed.

With somewhat similar ideas in mind, we attempted to determine the effect of adrenal hormones on the synthesis of mucopolysaccharides in the cock's comb.[25] As previously shown by Boas,[26] the cock's comb forms a large amount of polysaccharide, which is presumably hyaluronic acid. The quantity of this polysaccharide present in the comb is markedly increased by administration of androgens. We attempted to use this system to determine whether cortisone affected the synthesis of hyaluronic acid as measured by hexosamine content of both the total comb and of sodium acetate-extracted polysaccharide. No effect of cortisone was observed after either local or systemic administration. Similar results were obtained when the animals were pretreated with testosterone or treated simultaneously with both hormones.

Another group of experiments performed at the same time furnished further striking evidence regarding local specificity. Although the increased synthesis of hyaluronic acid under the influence of androgens was readily observed, simultaneous measurement of mucopolysaccharide formation in the skin of the same animals revealed no stimulation by androgen administration.* It is thus apparent that the inhibition of mucopolysaccharide synthesis under one set of circumstances does not permit generalization regarding other conditions.

Summary

A variety of reports have been concerned with the effect of adrenal hormones on connective tissues. Such investigations have frequently had as their basis an attempt to explain the striking effect of these hormones on "diseases of connective tissue." The results of these studies have indicated that the administration of cortisone under certain conditions influences proliferation and differentiation of connective tissue cells,[3-12] diffusion through connective tissue[27, 28] and, possibly, synthesis of chondroitin sulfuric acid.[24] At the present time, it is impossible to state whether these phenomena are based on the specific action of cortisone upon certain elements of connective tissue or are merely a reflection of the effects of cortisone upon certain basic biochemical or biophysical properties that are common to cells of various types. The effects of adrenal hormones upon the vascular system reported elsewhere in this symposium are of importance in interpreting the results of studies on connective tissue. In studies of such phenomena as the inflammatory reaction, it is not possible to determine at present whether connective tissue or vascular effects are primary. A consideration of the nature of connective tissue indicates that the state of connective tissue may be markedly influenced by materials entering it either from the capillaries or the parenchymal cells. The connective tissue barrier may in turn, however, play a role in regulating the flow of materials between the blood and parenchymal cells and the composition of intercellular fluid.

The behavior of connective tissues in specific areas does not permit generalization regarding the general behavior of connective tissue.

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* These unpublished experiments were performed by Dr. Sara Schiller.

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INTERACTION OF STEROID COMPOUNDS AND SINGLE CELLS*

I: MORPHOLOGICAL EFFECTS

By Lawrence M. Leonard and David D. Rutstein†

Department of Preventive Medicine, Harvard University Medical School, Boston, Massachusetts; and the Marine Biological Laboratory, Woods Hole, Massachusetts

Physiological and pharmacological effects of steroid hormones have been correlated with their chemical structure, but the mechanism of their interaction with the receptor cell is unknown. In these preliminary studies, techniques have been developed and data have been collected that provide an approach to the definition of this interaction.

For these experiments a single cell, the mature unfertilized egg of a marine annelid, *Chaetopterus pergamentaceus* (FIGURE 1), was chosen because it and similar marine eggs are readily available and are easily handled and photographed. Moreover, they have been extensively studied and their reactions to known chemical substances and to sea water of varying concentrations are well known.[1-12]

The first of these studies is concerned with the morphological appearance of this cell after exposure to progesterone, desoxycorticosterone, testosterone, 17-hydroxy-11-dehydrocorticosterone (cortisone, compound E) and 17-hydroxycorticosterone (compound F).‡

Method of Study

(*1*) *Stock Solutions.* Because of the low solubility of the steroids in sea water and since it had been determined previously that aqueous solutions could be prepared by addition of small amounts of alcoholic solutions of the crystalline steroids to water, stock solutions of each of the steroids under study were prepared in a concentration of 10 mg./ml. of 95 per cent ethanol.

(*2*) *Stock Egg Suspensions.* The eggs were obtained according to well-established procedures at the Marine Biological Laboratory at Woods Hole, Massachusetts, by mincing and filtering the ovaries from the parapodia of the worm.[13] The eggs were washed by repeated addition and decantation of freshly filtered sea water. To achieve comparability of egg dosage in repeated experiments, the concentration of eggs was adjusted to 0.05 ml. per 15 ml. of solution in a graduated stock dish. In each experiment, a single stock suspension from the parapodia of one worm was used. Throughout all experiments, the eggs were kept at sea water temperature (20 ± 0.5°C.). Utmost care was observed in the handling and in the transfer of the eggs to prevent their damage since eggs carelessly handled disintegrate after a few hours in sea water at 20°C.

(*3*) *Optical System and Photographic Technique.* The optical system consisted of a Bausch and Lomb Research Lamp, a Zeiss microscope with a 4× objective, a C.G. Grand photometer, a Leitz Micro-Ibso attachment with the

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† With the technical assistance of Jane H. McIntyre and C. Evans Roberts. Especial thanks are due to Katherine H. Hendrie who prepared the tables and the chart.

‡ Hereinafter referred to as cortisone and compound F., respectively.

Leitz 10× periplan ocular, and a 35 mm. Leica camera. A water cell with heat-absorbing glass was placed between the light source and the substage condenser to reduce to a minimum the heating effect of the light on the solutions. A light-green Wratten filter was placed in the system to achieve as nearly monochromatic light as possible. The intensity of the light illuminating the eggs was kept constant to prevent variation in size of the image on the negative. This was assured by focusing the microscope on the bottom of an optical-glass dish containing 10 ml. of sea water, adjusting the lamp and substage condenser for Köhler illumination,*[14] and by then regulating the intensity of the light by means of the substage diaphragm alone until the photometer (which registers the total flux of light, *i.e.*, intensity multiplied by the area of the field) indicated the desired value. This value had been determined previously by trial and error as the best flux of light with a shutter speed of 1/125th of a second to produce a "good" negative on Kodak Panatomic-X film with normal development in Kodak Microdol. When each dish with its partially opaque eggs was placed on the stage, the flux was thereby decreased, but the intensity of light illuminating each egg remained the same and reproducible negatives were obtained.

When the negative is enlarged with constant exposure and development, the image of the egg on the photographic paper, when measured with a planimeter, will then be of constant size.[15] The magnification of each series of enlargements was checked on a print similarly made of a photograph of a stage micrometer (FIGURE 2), in which the main divisions (as from 1.3 to 1.4) were 0.1 mm. (100 μ). The diameters of the eggs in a given series of enlargements may also be checked by comparing the scale with the photomicrographs of the eggs in the control pictures (approximately 100 μ).

All enlargements were made on double-weight Kodabromide projection paper, contrast No. 2, surface DL (semi-matte), which provides a good base for the planimeter. The prints were dried in a 100-watt Lott rotary drier, which did not affect the size or shape of the photographic image.

(4) *Experimental Procedure.* In each series of experiments, the optical-glass dishes were filled with 10 ml. of freshly filtered sea water and placed in a bath at 20 ± 0.5°C. The first dish or each series was the "sea water control." To the second dish, the "ethanol control," 0.1 ml. of 95 per cent ethanol was added. In the dishes containing hormone, when a final concentration of 0.1 mg./ml. was required, 0.1 ml. of stock hormone solution containing 10 mg./ml. was added to the 10 ml. of sea water. When a concentration less than 0.1 mg./ml. was desired, the appropriate amount of stock solution was used and enough 95 per cent ethanol added to achieve a final ethanol volume of 0.1 ml. In every experiment, the last two dishes comprised another set of controls, one "sea water control" and one "ethanol control."

The eggs were transferred in 15 ml. aliquots with a medicine dropper from the stock dish to centrifuge tubes. These were slowly spun down in a hand centrifuge and the supernatant carefully decanted. The eggs were then gently poured into the dishes and immediately examined microscopically for visible damage. If more than a few eggs were disintegrated, the suspension was discarded and a new one prepared.

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* Köhler illumination was used to produce a sharp image and even field illumination.

Throughout the experiment, the eggs were carefully observed through the side telescope of the Micro-Ibso attachment so that, when the morphology changed, photographs could be taken. The eggs in the other experimental and control dishes were photographed as soon as possible thereafter. Those experiments in which no morphological changes occurred were observed frequently and photographs taken at specified intervals. Most of the experiments were terminated at six hours, although a few comparisons were made at twenty-four hours.

FIGURE 1. *Chaetopterus pergamentaceus.*

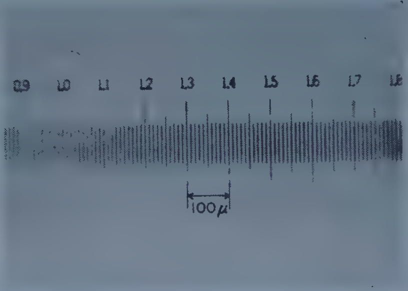

FIGURE 2. Photomicrograph of stage micrometer illustrating magnification in subsequent FIGURES except 4b and 5b.

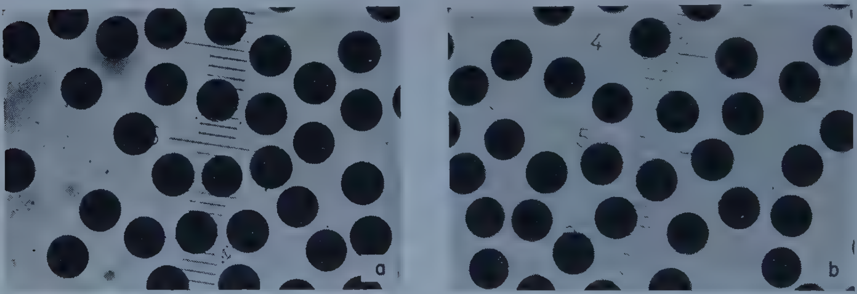

FIGURE 3. Photomicrographs of eggs of *Chaetopterus pergamentaceus* in control solutions. (3a) Sea-water; (3b) 0.1 ml. of 95 per cent ethanol in 10 ml. of sea-water.

It is to be noted that the concentrations of hormones in these experiments are greater than those usually required for physiological response in experiments on intact animals and that the role of steroid hormones in the normal metabolism of this cell is unknown.

Results

I. Effects from 0 to 6 Hours

(*A*) *Controls.* Seventy-four "sea water controls" were run in conjunction with the experimental dishes. Of these, 63 were unchanged at six to twelve hours (FIGURE 3a), and the remaining 11 were unchanged for three to five hours and were not further observed. Seventy-seven "ethanol controls" were run, with 64 of these unchanged at six to twelve hours (FIGURE 3b) and the other thirteen were unchanged at three to five hours and were not further observed. Many experiments were discarded where the eggs in the "sea water control" or "ethanol control" had disintegrated in less than six hours.

TABLE 1

PROGESTERONE DILUTION SERIES ON *Chaetopterus pergamentaceus* EGGS AT SIX HOURS

Progesterone, mg./ml.	Per cent normal		Progesterone, mg./ml.	Per cent normal	
	Exp. 1	Exp. 2		Exp. 1	Exp. 2
0*	90	70	0.01	80	73
0†	87	74	0.03	71	—
0.001	70	75	0.05	14	6
0.003	84	—	0.08	10	—
0.005	75	—	0.1	10	—
0.008	78	—			

* "Sea-water control."
† "Ethanol control."

TABLE 2

SAMPLE EXPERIMENT. PROGESTERONE EFFECTS ON *Chaetopterus pergamentaceus* EGGS BY DURATION OF EXPOSURE AND CONCENTRATION

Duration		Concentration	
Hrs.	Min.	0.05 mg./ml.	0.1 mg./ml.
0	5	Normal	Normal
0	6	—	Crenation and small lifted membranes
0	12	—	Membranes lifting and ghosts forming
0	20	Few crenated	—
1	0	Much crenation, few buds, few membranes lifting	—
1	30	—	Almost all with lifted membranes or forming ghosts
2	40	All crenated, budded and/or with with lifted membranes	—
3	30	—	Membranes lifted, contents vacuolated; many ghosts present
6	0	Membranes lifted, ghosts	—

(*B*) *Progesterone.* Two preliminary experiments were done at a concentration of 0.1 mg./ml. of progesterone. The morphology of the eggs in these dishes changed too fast for precise observation. Two experiments were then done to obtain the concentration that produced a definite series of effects, which could be followed conveniently.* TABLE 1 summarizes the results. At a concentration of 0.03 mg./ml. or less, a very small percentage of the eggs are affected, while at a concentration of 0.05 mg./ml. or greater, three-fourths are affected at six hours. The rate at which qualitative changes occurred in concentrations of 0.05 mg./ml. and 0.1 mg./ml. are contrasted in TABLE 2. The 0.05 mg./ml. concentration was selected and 26 experiments were performed at this concentration with a distinctive pattern of effects in all. The course of the reaction is: crenation of the membrane (FIGURE 4a), followed by a budding (FIGURE 4b),* then a separation of the membrane from the egg (FIGURE 4c) and, ultimately, in many eggs the extrusion of the protoplasm through the membrane to produce "ghosts" (FIGURE 4d, TABLE 2). After the membrane crenates, the eggs sometimes appear vacuolated. The time of crenation of the membrane varied in the different experiments from five to sixty minutes, the large majority crenating in thirty minutes (CHART 1). The time for the separation of the membrane varied from forty to ninety minutes. By six hours, 90 per cent or more of the eggs had membranes separated from the egg or had become ghosts. In all of these progesterone experiments the double set of controls appeared normal at six hours.

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* These two experiments are the only ones reported where eggs in the control solutions showed changes prior to six hours. In the control solutions, less than one-fourth of the eggs were affected and these do not obscure the striking changes in the eggs in the higher progesterone concentrations.

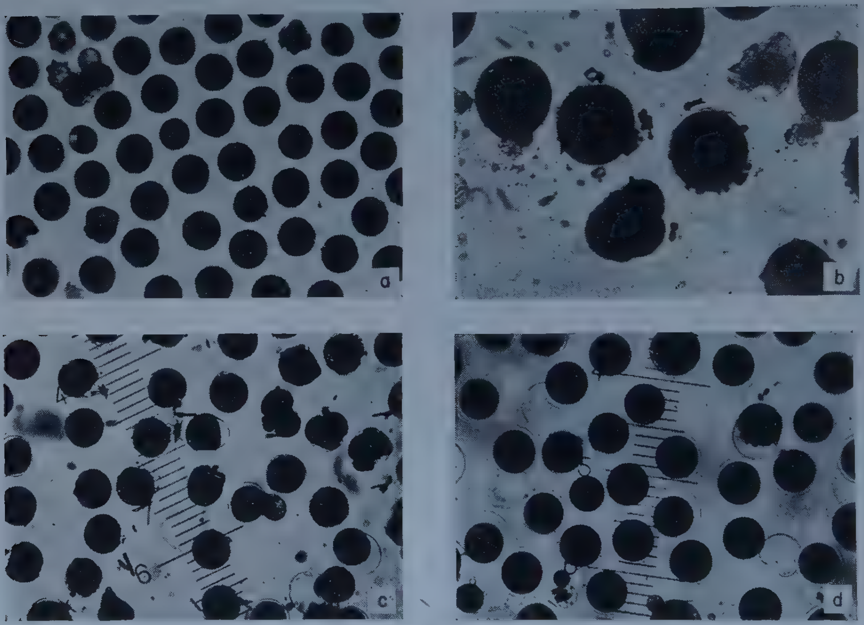

FIGURE 4. Photomicrographs of eggs of *Chaetopterus pergamentaceus* in solutions of progesterone in sea-water. (4a) 0.05 mg./ml. at 75 minutes; (4b) 0.05 mg./ml. at 2 hours (magnification is twice that of FIGURE 4a); (4c) 0.1 mg./ml. at 30 minutes; (4d) 0.1 mg./ml. at 6 hours.

(*C*) *Desoxycorticosterone.* Two preliminary experiments were done with 0.1 mg./ml. of desoxycorticosterone. Eggs in the dishes with desoxycorticosterone produced a crenation of the membrane in one to two hours, a very slow reaction compared with the eggs in progesterone. Two dilution experiments (TABLE 3) showed at six hours that, at concentrations of 0.03 mg./ml. or less, a small percentage of the eggs were affected; at 0.05 mg./ml., approximately half were changed; and at 0.08 mg./ml., most of the eggs showed morphological changes. Although 0.08 mg./ml. could have been used in subsequent experiments, the more convenient concentration of 0.1 mg./ml. was finally selected and used in a total of 21 experiments. The course of reaction was followed in 11 of these; in the other 10, only control and final observations were made. In two of the 11 experiments, a crenation of the membrane (FIGURE 5a) was noted within thirty minutes and, in the other nine, the time for crenation varied between one and two and one-half hours (CHART 1). The eggs then showed small

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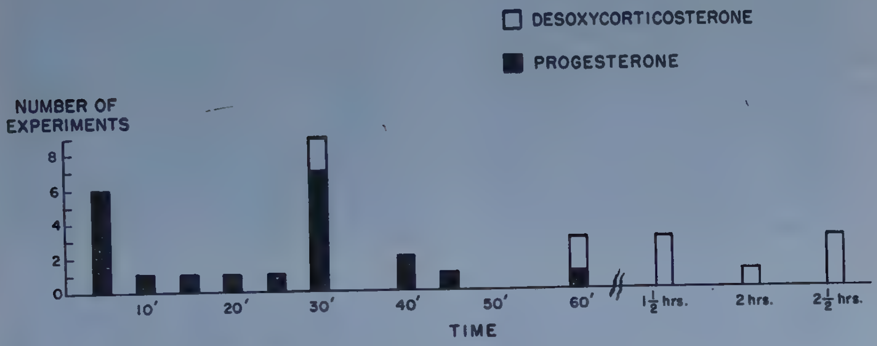
* The magnification of FIGURE 4b is twice that of FIGURE 4a.

PROGESTERONE (0.05 mg/ml) VS. DESOXYCORTICOSTERONE (0.1 mg/ml)
TIME OF INITIAL CRENATION OF EGGS OF CHAETOPTERUS PERGAMENTACEUS BY NUMBER OF EXPERIMENTS*



*Time of initial crenation was not noted in 5 of 26 progesterone and 10 of 21 desoxycorticosterone experiments.

TABLE 3

DESOXYCORTICOSTERONE DILUTION SERIES ON *Chaetopterus pergamentaceus* EGGS AT SIX HOURS

Desoxycorticosterone mg./ml.	Per cent normal	
	Exp. 1	Exp. 2
0*	100	100
0†	100	100
0.001	—	100
0.008	—	100
0.01	99	99
0.03	92	90
0.05	55	37
0.08	0	0
0.1	0	0
0†	100	100
0*	100	100

* "Sea-water control."
† "Ethanol control."

protrusions of the membrane (FIGURE 5b*) which were different from those produced by the progesterone (FIGURE 4b). In two early experiments these protrusions looked like tiny fountains (FIGURE 5c) and were different from any of the changes occurring with any of the other hormones tested. In about one-half of the experiments the eggs budded after the protrusions. At six hours many of the eggs have raised membranes or have become ghosts in a concentration of 0.1 mg./ml. of desoxycorticosterone (FIGURE 5d, TABLE 4), but not to the extent of the eggs in 0.05 mg./ml. of progesterone. CHART 1 illustrates the striking difference between progesterone† and desoxycorticosterone in the time required for initial morphological change, *i.e.*, crenation. In both the progesterone and desoxycorticosterone experiments, the rate of effect and the proportion of eggs affected varied directly with the concentration of the hormone.

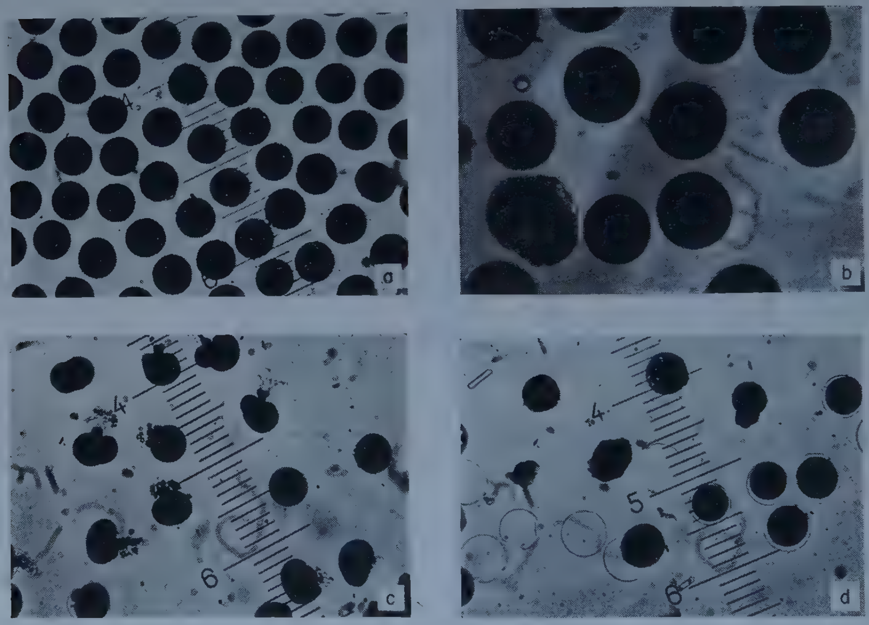


FIGURE 5. Photomicrographs of eggs of *Chaetopterus pergamentaceus* in solutions of desoxycorticosterone in sea-water. (5a) 0.1 mg./ml. at 45 minutes; (5b) 0.1 mg./ml. at 2 hours (magnification is twice that of FIGURE 5a); (5c) 0.1 mg./ml. at 3 hours; (5d) 0.1 mg./ml. at 6 hours.

(*D*) *Testosterone.* This steroid was tested at two different concentrations, 0.05 and 0.1 mg./ml. (TABLE 5). The reaction to 0.05 mg./ml. of testosterone was slow and at the end of six hours, only a few of the eggs were affected. Eighteen experiments were done with 0.1 mg./ml. of testosterone. In 12 of these the course of reaction was noted, while in the other six only initial and final observations were made. In seven of the 12, observations were made in 45 to 90 minutes from the start of the experiment, and in all of these crenation was present (FIGURE 6a). In all 18 experiments, the eggs showed, during the six hour experiment, a unique type of budding characteristic of exposure to testosterone (FIGURE 6b). In contrast to progesterone and desoxycorticosterone, neither raised membranes nor ghosts were produced by testosterone at any time during the six-hour experiment.

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* The magnification of FIGURE 5b is twice that of FIGURE 5a.

† The time of initial crenation was noted in 21 of the 26 experiments using 0.05 mg./ml. progesterone.

TABLE 4

SAMPLE EXPERIMENTS. DESOXYCORTICOSTERONE EFFECTS AT CONCENTRATION OF 0.1 MG./ML. ON *Chaetopterus pergamentaceus* EGGS BY DURATION OF EXPOSURE

Duration		Experiment	
Hrs.	Min.	No. 1	No. 2
0	40	Normal	Normal. Immature eggs are present
1	0	Few crenated	—
2	0	⅓ budded; rest crenated with membrane protusions	Most slightly crenated; several budded
3	0	⅓ budded; many crenated with membrane protusions; few ghosts	—
3	30	—	Most crenated; few irregular
6	0	Some buds; lifted membranes; many ghosts	—
6	30	—	All crenated with membrane protusions

TABLE 5

SAMPLE EXPERIMENT. TESTOSTERONE EFFECTS ON *Chaetopterus pergamentaceus* EGGS BY DURATION OF EXPOSURE AND CONCENTRATION

Duration		Concentration	
Hrs.	Min.	0.05 mg./ml.	0.1 mg./ml.
0	24	Normal	Normal
0	26	—	Minimal crenation
0	42	Minimal crenation	—
1	20	—	Minimal crenation
2	0	—	All slightly crenated; ¼ with small clear buds
2	30	Minimal crenation	—
6	0	Few budded without lifted membranes; rest normal	Most distended with crenation on one end; rest budded without lifted membranes.

These three hormones, progesterone, desoxycorticosterone, and testosterone, produce morphological changes in the egg of *Chaetopterus pergamentaceus*. They can be contrasted with the following hormones, which did not produce morphological changes at six hours.

(*E*) *Cortisone.* Nine experiments were performed with 0.1 mg./ml. of cortisone. During the period of six hours, the eggs appeared unchanged (FIGURE 7a) and could not be distinguished from those in the control solutions.

(*F*) *Compound F.* Eight experiments were done with 0.1 mg./ml. of Compound F. In six, the eggs were unchanged and could not be distinguished from the controls. In the other two most of the eggs appeared unchanged though a very few eggs showed marked budding (FIGURE 7b). This field was chosen to show the budding. Most of the other fields showed no change and looked like FIGURE 7a. Five experiments were done with 0.05 mg./ml. of Compound F with the eggs showing no change at six hours.

Note: Estradiol, prepared in concentration of 10 mg./ml. of 95 per cent ethanol, precipitated immediately when 0.1 ml. was added to 10 ml. of sea water. The eggs in this suspension of sea water and estradiol showed no change over a period of six hours, but, because so little remained in solution, no conclusions are drawn from this experiment.

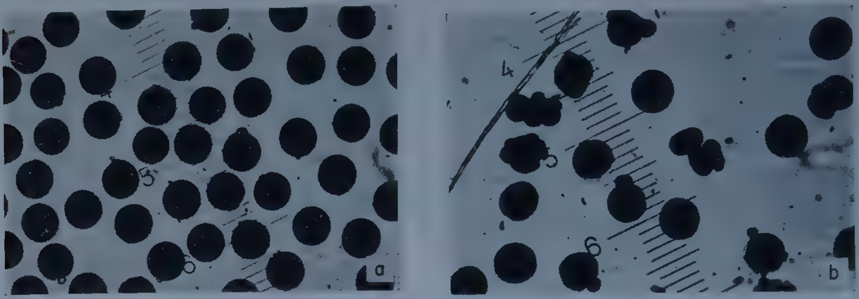

FIGURE 6. Photomicrographs of eggs of *Chaetopterus pergamentaceus* in solutions of testosterone in sea-water. (6a) 0.1 mg./ml. at 90 minutes; (6b) 0.1 mg./ml. at 3 hours.

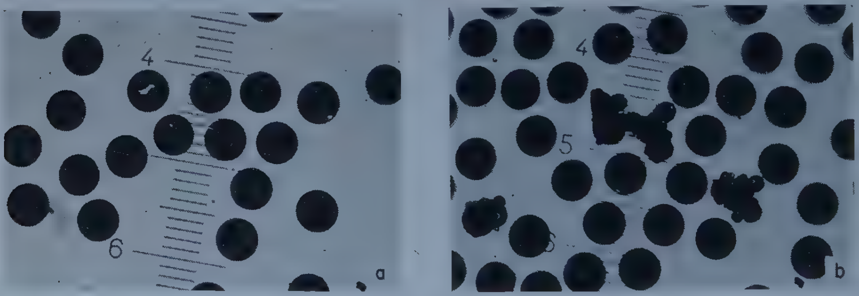

FIGURE 7. Photomicrographs of eggs of *Chaetopterus pergamentaceus* in solutions of 17-hydroxy-11-oxysteroids in sea-water. (7a) 0.1 mg./ml. cortisone at 6 hours; (7b) 0.1 mg./ml. compound F at 6 hours.

II. Effects at 24 Hours

At 24 hours, the eggs in progesterone could not be distinguished from those in desoxycorticosterone, since raised membranes and ghosts were present in both, though not in the controls. Testosterone effects could easily be distinguished from those of progesterone, desoxycorticosterone, and the controls, because of the marked budding and lack of separated membranes or ghosts.

The eggs in the "sea water control" (FIGURE 8a) and "ethanol control" (FIGURE 8b) for 24 hours were in process of slow disintegration.

In all of the nine experiments performed, the eggs in cortisone (FIGURE 8c) were much less disintegrated at 24 hours than those in either the "sea water control" (FIGURE 8a) or in the "ethanol control" (FIGURE 8b). In four experiments performed with compound F (FIGURE 8d), the eggs were noticeably less disintegrated than the eggs in the "sea water control," or in the "ethanol control." In two of these, this effect was as great as in cortisone and, in the other two, it was less.

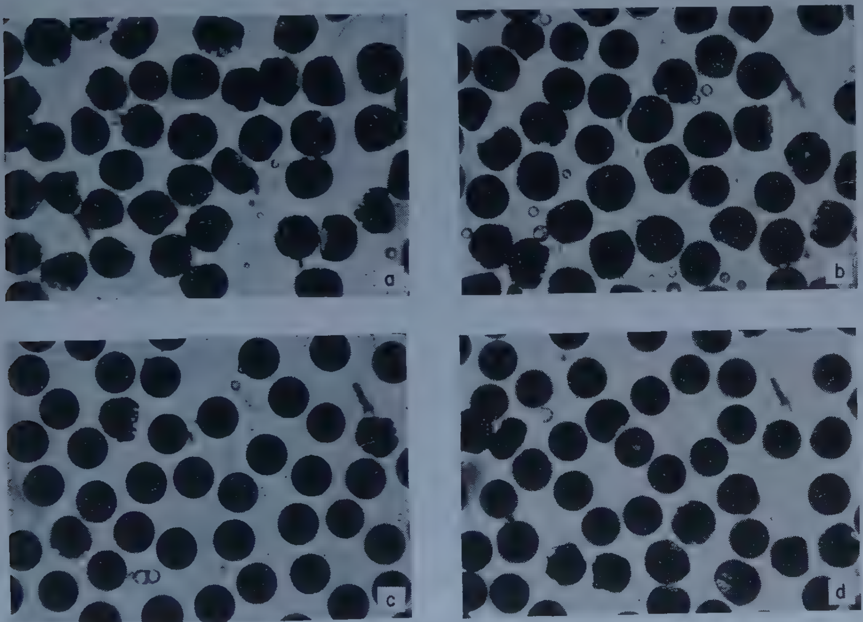

FIGURE 8. Comparison of *Chaetopterus pergamentaceus* eggs at 24 hours in control and 17-hydroxy-11-oxysteroid solutions. (8a) "Sea-water control;" (8b) "Ethanol control;" (8c) 0.1 mg./ml. cortisone; (8d) 0.1 mg./ml. compound F.

Discussion

The morphological changes produced in the egg of *Chaetopterus pergamentaceus* by progesterone, desoxycorticosterone, or testosterone are striking and distinctive. They provide the evidence for a specific reaction between each of these hormones and the cell. They also suggest that this egg may be used as an indicator of the presence of these hormones.

The lack of any morphological change and the maintenance of the "normal" appearance of the cell after exposure to the 17-hydroxy-11-oxysteroids (cortisone and compound F) is in striking contrast to the changes produced by progesterone, desoxycorticosterone, or testosterone. This lack of morphological change does not indicate that cortisone and compound F do not react with the cell. The more "normal" appearance of cells in solutions containing the 17-hydroxy-11-oxysteroids in comparison with control eggs in sea water at twenty-four hours suggests that these compounds prevent morphological change, and that they assist in the maintenance of the integrity of the cell.

These data, if supplemented by study of other steroid compounds by this same technique, should be useful in determining possible correlations between the chemical structure of the steroid molecule and specific morphological changes in the cell.

Summary and Conclusions

(1) The mature unfertilized egg of *Chaetopterus pergamentaceus* undergoes specific and distinctive morphological changes after exposure to progesterone, desoxycorticosterone, or testosterone, and maintains its normal appearance after exposure to cortisone or compound F.

(2) This cell maintains its morphological appearance better at 24 hours in sea water containing cortisone or compound F than in sea water not containing one of these 17-hydroxy-11-oxysteroids.

(3) The significance of these findings is discussed.

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INTERRUPTION BY TOPICAL CORTISONE OF LEUKOCYTIC CYCLES IN ACUTE INFLAMMATION IN MAN

By John W. Rebuck and Raymond C. Mellinger

Departments of Laboratories and Medicine, The Henry Ford Hospital, Detroit, Michigan

Study of the mechanisms of the remarkable effects of cortisone and adrenocorticotropic hormone on inflammation was materially advanced by the reports of Steffensen and his associates[1, 2] that the application of topical cortisone, in patients suffering with inflammatory disease of the oculus, produced anti-inflammatory effects. The clinical implications of this finding have been repeatedly confirmed and expanded.[3, 4, 5, 6, 7, 8, 9, 10] Experimental exploration of possible mechanisms of topical corticosteroid action has been less extensive. Blocking of the inflammatory phase of the reactions induced by the injection of jequirity or horse serum (Woods[3]) or by jequirity infusion or staphylococcus toxin (Woods and Wood[11]) when cortisone was injected locally has been reported. Leopold and his co-workers[9] reported comparable inhibition of the inflammation produced with *Aerobacter aerogenes*, *Escherichia coli*, or *Proteus vulgaris*. Consideration of possible mechanisms of local cortisone action at a cellular level was given by Castor and Baker,[12] who observed a resultant decrease in the number of dermal fibroblasts; by Dougherty,[13] who counted the number of damaged fibroblasts and nonautochthonous cells as a measure of cortisone inhibition of the subcutaneous inflammation produced by histamine in adrenalectomized mice; by Shapiro, Taylor, and Taubenhaus,[14] who noted that cortisone in a turpentine abscess produced a more necrotic leukocytic barrier and thinner granulation tissue than did turpentine alone in rats, and that ischemia or denervation did not interfere with such inhibitory effects of cortisone; and by Benjamin, Cornblatt, and Grossman,[15] who noted "less infiltration" in their human biopsy specimens six hours after intradermal injection of adrenocortical hormones. The above observations are to be distinguished from the more extensive consideration afforded the focal action of parenterally administered ACTH and cortisone by many workers in the field whose findings will be discussed below.

With attention pertaining to the mechanism of topical cortisone inhibition of inflammation so centered at a cellular level, further inquiry into the nature of such mechanisms requires a proper appreciation of the leukocytic cycles that play their part in the dynamic process that is inflammation. The basic cellular cycles of acute inflammation were first fully described by Metchnikoff[16, 17] in experimental animals. They consisted of migratory cycles of phagocytic polymorphonuclear leukocytes and lymphocytes, which latter hypertrophied to form macrophages (large phagocytic cells). These white blood cells and their derivatives were reinforced by the blood mononuclears, later called monocytes, and by tissue macrophages, later fully described by Aschoff and Kiyono[18] as histiocytes. This delineation of inflammatory leukocytic dynamics in experimental animals has been confirmed by Maximow,[19] Downey,[20] Bloom,[21] Watson,[22] Taliaferro and Mulligan,[23] Kolouch,[24] and many others. Full reaffirmation of these basic cellular concepts of Metchnikoff has been afforded in the last decade by a number of workers utilizing modern and advanced techniques in the study of rabbit-ear window,[25] the central nervous system,[26, 27, 28] in connective tissue spreads stained like blood smears,[29, 30] in both dry films[31] and motion pictures[32] of tissue cultures, in the study of individual living leukocytes,[33] and in human-skin windows.[33, 34, 35]

The problem of the cellular mechanism of the inhibitory effect of parenterally (systemically) administered cortisone upon local inflammation in adrenalectomized mice was resolved by Dougherty and Schneebeli,[30] who demonstrated marked diminution of polymorphonuclear leukocytic participation during the first stages of inflammation, and decreased participation of macrophages during the later stages. Rebuck, Smith, and Margulis[35, 36, 37] similarly demonstrated far-reaching qualitative and quantitative cellular defects in acute inflammatory exudates in patients treated with parenterally administered ACTH. In the early stages of neutrophilic leukocytic predominance, the latter authors demonstrated significant depression of neutrophilic phagocytic activity, diminished lymphocytes and their hypertrophied forms at intermediate stages and, finally, the resultant impairment of phagocytic potentials as the consequence of near deletion of the important mobile source of the macrophages. It is the purpose of this paper to examine the problem of the cellular mechanism of the inhibitory effect of topically administered cortisone upon acute inflammation in man.

Materials and Methods

A series of permanent, fixed preparations of *in vivo* samplings of the cellular exudates were obtained in human volunteers with experimental inflammatory lesions. The lesions were prepared by scraping away the epithelium from a small area of the skin on the volar surface of the forearm. In addition to the trauma of the technique, which served as the primary inflammatory excitant, a nonpyogenic antigen (diphtheria toxoid) was applied, and the lesion was covered with a chemically clean, sterile cover slip. The time at which the epithelium was removed marked the time inflammation began. Within an hour, the cells of the inflammatory exudate had migrated to the under surface of the cover slip. When such migration had been effected, the cover slip was removed and additional cover slips were placed over the same lesion at timed intervals. The series of cover slips collected from each lesion were air-dried and stained like blood smears. The May-Grünwald-Giemsa staining procedure was employed in the experiments to be described. This technic has been reported previously[33, 35, 38] and lends itself to electron microscopy[39] and histochemical analysis[40, 41] when desired.

Preliminary lesions were applied and followed in 11 adult volunteer controls not subjected to treatment. Cover slip preparations obtained at 2, 3, 4, 5, 6, 7, 9, 12, 14, 15, 16.5, 18, 20, 21, 22, 23, and 24 hours were studied in order to establish the basic cellular sequences of acute inflammation in man.

Six series of experimental lesions were similarly studied with the additional application of topical cortisone at various stages in the development of the leukocytic cycles. In two series, 11-dehydro-17-hydroxy-corticosterone in the acetate form (Cortone Acetate, Merck)* was applied as a powder suspended in homolgous serum. In the remaining four series, a microcrystalline suspension of cortisone acetate (Cortone Acetate, Merck), 25 mg. per cc. in normal saline, with suspending agents and 1.5% benxyl alcohol as a preservative was utilized. Preparations were obtained at 3, 3.5, 4, 5, 6, 7, 9, 10, 11.25, 12, 13.5, 14, 14.75, 24, 25, and 26.5 hours of inflammation. An untreated lesion, 5 cm. distal to the experimental lesion and therefore on the side opposite to the direction of its venous and lymphatic drainage, served as an additional control for each series. In the latter, control lesions, equal amounts (0.05 cc.) of homologous serum without cortisone, normal saline, or the suspending agents and 1.5% benzyl alcohol† were applied topically at the same times as topical cortisone was applied to the experimental lesions.

Phagocytic activity of the exudative cell types was tested for by application of small amounts of India ink to the denuded surface of both experimental and control lesions at timed stages in the inflammatory cycle.

Cytology of Control Lesions. Study of the series of preparations obtained from the 11 adult volunteers not subjected to treatment and from the controls lesions paired with experimental lesions revealed normal leukocytic cycles comparable to the basic patterns delineated by Metchnikoff, as described above. For the first eight to ten hours after removal of the epidermis, increasing numbers of neutrophilic leukocytes were found within the lesions. Small numbers of tissue histiocytes, monocytes, and lymphocytes were observed during the same period. When first observed, the neutrophils were swollen, but their measurements returned rapidly to normal and, thereupon, the neutrophilic size decreased steadily until, by 14 hours of inflammation (FIGURE 5), neutrophilic diameters were in the 6 to 8 micra range. Loss in neutrophilic cell size was accomplished by disintegration or budding of the specific granule-containing cytoplasm to the exudative fluids,[34] and by gradual pyknosis and shrinkage of nuclear lobes. By 14 hours of inflammation, in the simple cycles to which neither control nor active test materials were added, neutrophils comprised less than half of the cells present in the preparations. Introduction of India ink at any stage in the inflammatory process produced a second wave of neutrophilic migration and rendered the nonpyogenic response to the original antigen (diphtheria toxoid) pyogenic in nature (FIGURE 1).

Lymphocytic participation in the control lesions became conspicuous by the 10th hour of inflammation. After 12 hours of inflammation, numerous hypertrophying lymphocytes with 0.5 to 1.0 micron additional cytoplasmic mass were present in addition to lymphocytes which still retained the size and structural characteristics of lymphocytes in the blood. After 14 hours, lymphocytes and their hypertrophying forms were the predominant cell type (FIGURE 5). From 14 to 24 hours further, hypertrophy eventuated in the predominance of histiocytes. During this stage, the hypertrophying (lymphocytogenous) forms approached the size and structure of the tissue histiocytes. Tissue histiocytes

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* The 11-dehydro-17-hydroxycorticosterone in the acetate form (Cortone Acetate, Merck) was made available through the courtesy of Dr. J. M. Carlisle, Merck Laboratories, Rahway, N. J.

† We are indebted to Dr. E. Alpert, Merck and Co. for the aqueous vehicle for cortisone used as a control preparation.

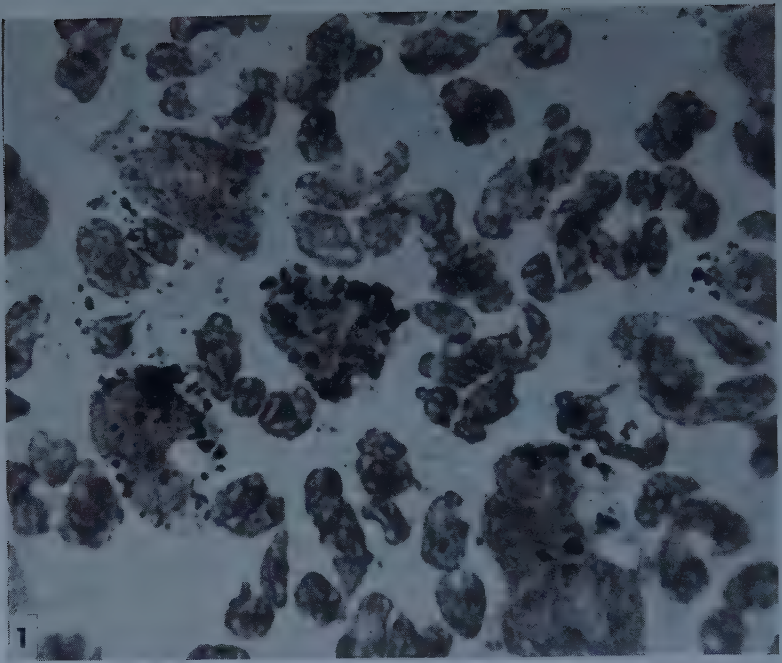

FIGURE 1. 12 hrs. of inflammation. Untreated control. Previous topical application of physiological saline and India ink. Moderate phagocytosis of India ink by lymphocytes and neutrophils. × 1800.

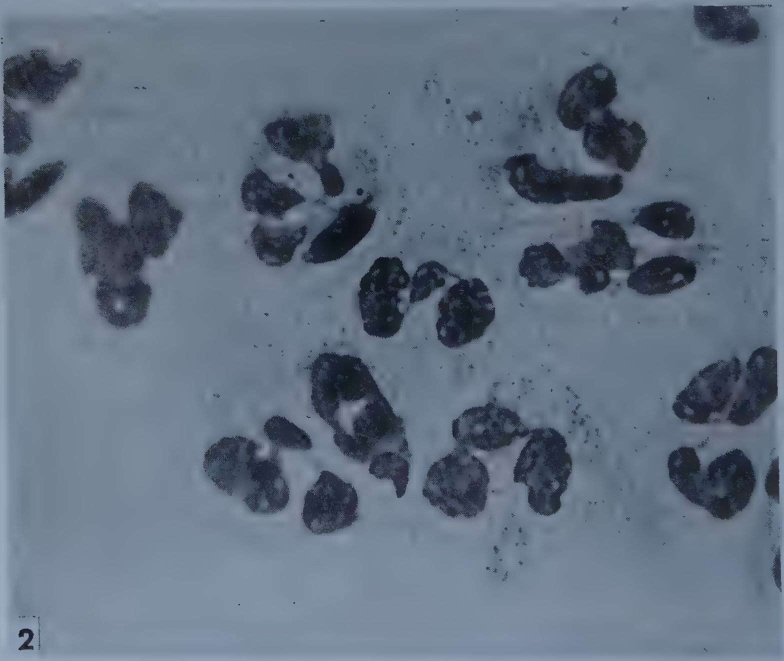

FIGURE 2. 12 hrs. of inflammation in same patient as FIGURE 1. Previous application of topical cortisone and India ink. Sparse neutrophilic phagocytosis. Note lymphocytic hiatus here. × 1800.

were present in relatively small numbers during all phases of the inflammatory response. In judging cell size, it is well to keep in mind the exact magnification employed or, better still, to employ a micron scale in the manner of the electron microscopists because, hour by hour as the neutrophils decreased in size, the lymphocytes added steadily to their cytoplasmic mass. Monocytes were present in small numbers throughout the inflammatory cycle (TABLE 1) and were easily distinguished from lymphocytes and hypertrophying lymphocytes because the monocytes in the preparations under discussion, stained like blood smears, revealed the same identifying structural characteristics to be found in their counterparts in the peripheral blood. This latter finding, of numerically minor monocytic participation in the leukocytic cycles in inflammation in these subjects, is in agreement with the observations of those workers studying inflammation in animals or man with high peripheral blood monocytoses,[42, 43, 44] namely, that blood monocytes shared in the local defensive processes in a degree approximately proportional to their numbers in the peripheral blood that were available for migration.

TABLE 1

ILLUSTRATIVE DIFFERENTIAL EXUDATIVE CELLS COUNTS (1,000 CELLS)

	Topical saline		Topical cortisone		Aqueous vehicle	Topical cortisone	Aqueous vehicle		Topical cortisone	
	Phagocytic	Non-phagocytic	Phagocytic	Non-phagocytic			Phagocytic	Non-phagocytic	Phagocytic	Non-phagocytic
Experiment	E-103		E-102		E-105	E-104	E-105		E-104	
Hour of inflammation	12		12		14	14	24		24	
Lymphocytes	19	14	9	15	181	17	34	47	3	5
Hypertrophying lymphocytes	32	28	3	20	165	15	64	43	6	10
Macrophages (histiocytes)	38	43	7	32	191	24	133	67	17	14
Monocytes	23	14	0	31	13	4	6	8	0	2
Neutrophils	54	734	6	877	450	939	83	513	22	921
Totals	166	834*	25	975	1000	1000†	320	680‡	48	952

* One eosinophil observed.
† One basophil observed.
‡ Two eosinophils observed.

In four control lesions phagocytic activity of the participating cells was tested for by application of small amounts of India ink. Such studies were made at 10, 12, and 24 hours of inflammation. Appreciable numbers of neutrophils, lymphocytes, and histiocytes were capable of phagocytic activity, as were monocytes when present (TABLE 1). Lymphocytes were capable of ingesting the particulate test material (FIGURE 1) even when their cell bodies were no larger than those that they possessed in the blood stream but, in general, even small increases in lymphocytic cytoplasmic mass resulted in enhanced phagocytic activity, a finding previously demonstrated *in vitro* by Hertzog.[45] Although the application of India ink to the lesions interposed a predominantly pyogenic response, participation of lymphoid elements persisted to a degree

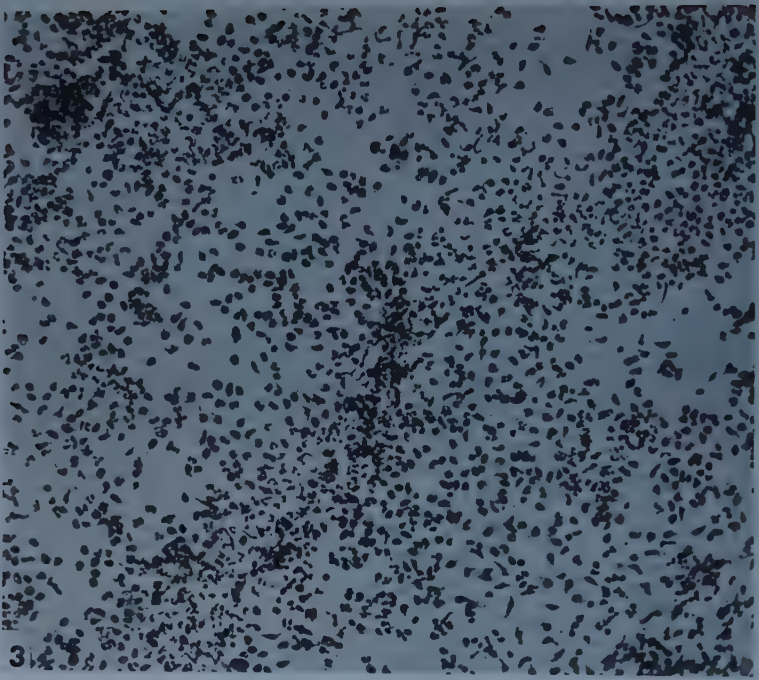

FIGURE 3. 14 hrs. of inflammation. Untreated control. Previous application of aqueous vehicle. Marked cellularity of exudate. × 130.

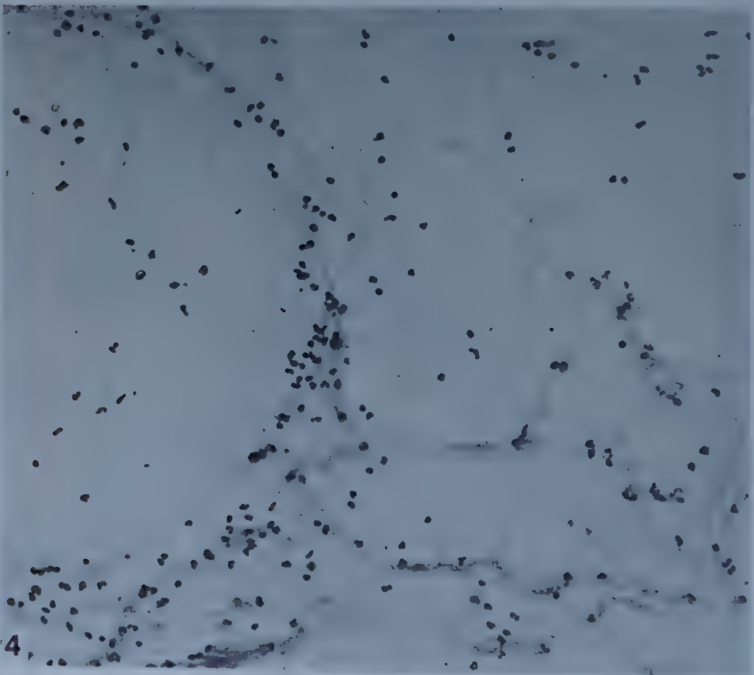

FIGURE 4. 14 hrs. of inflammation in same patient as FIGURE 3. Previous application of topical cortisone. Note paucity of exudative cells. × 130.

that permitted a statistically significant comparison of the effects of test materials upon the phagocytic abilities of such lymphoid elements (FIGURES 1 and 2, TABLE 1).

Mitoses were never observed in the participating cell types.

Cytology of lesions treated with topical cortisone. Experimental lesions were prepared in the same way as the control member of each pair. They were allowed to progress for varying times before application of cortisone. In the first two series, only a single topical application of the cortisone was employed; in a third series, two topical applications were made; and, in a fourth series, topical applications were repeated at three different stages in the development of the inflammatory cycles. In the first two series, the inflammatory process was permitted the same uninterrupted response to the initial trauma and diphtheria toxoid as was the control until seven hours had elapsed. After seven hours, in both the experimental and controls members of the series, there was a decidedly cellular exudate. Neutrophilic leukocytes were the most numerous among the cells present, although a few lymphocytes and tissue histiocytes were also observed at this time. After seven hours, 0.05 cc. of the microcrystalline suspension of cortisone acetate in its aqueous vehicle, containing suspending agents and 1.5 per cent benzyl alcohol, was applied to the denuded surface of the experimental lesions. At the same time, a comparable amount of physiological saline was applied to the control lesion. After ten hours of inflammation in the experimental lesions, or three hours after the application of the topical cortisone, there was definitely less exudate than in the control lesion or than had been present three hours previously in the experimental lesions themselves. All the cell types in the exudative response were decreased in numbers. The sparse cells remaining consisted of some swollen neutrophilic leukocytes, a few lymphocytes, and an occasional tissue histiocyte. The picture in the experimental lesions at this stage was in striking contrast to the rich cellular exudate in the control lesions at the same hour of inflammation for, in the control lesions, there were numerous neutrophilic leukocytes, a number of tissue histiocytes, and occasional lymphocytes. After 12 hours of inflammation in the experimental lesions which, in these first two series, were not subjected to any further treatment with topical cortisone, there occurred an excellent recovery of the cellular exudative response. There were many more cells than had been present two hours previously, and the cellularity of the experimental lesions compared favorably with that of the controls of the same stage. Neutrophilic leukocytes, lymphocytes, and hypertrophying lymphocytes made up the bulk of the cell types present; and some histiocytes were also observed. In the subsequent 14-hour and 24-hour stages, the experimental lesions went on to lymphocytic and hypertrophying lymphocytic predominance, which gave way ultimately to the final histiocytic picture present also in the controls. The single application of topical cortisone after seven hours of inflammation in man had interrupted the leukocytic responsive cycles for a few short hours, but they had been resumed in an almost normal fashion thereafter.

In a third series, the inflammatory process was again permitted an uninterrupted response to the initial trauma and diphtheria toxoid as was the control until seven hours had elapsed, and the first leukocytic migrations had been

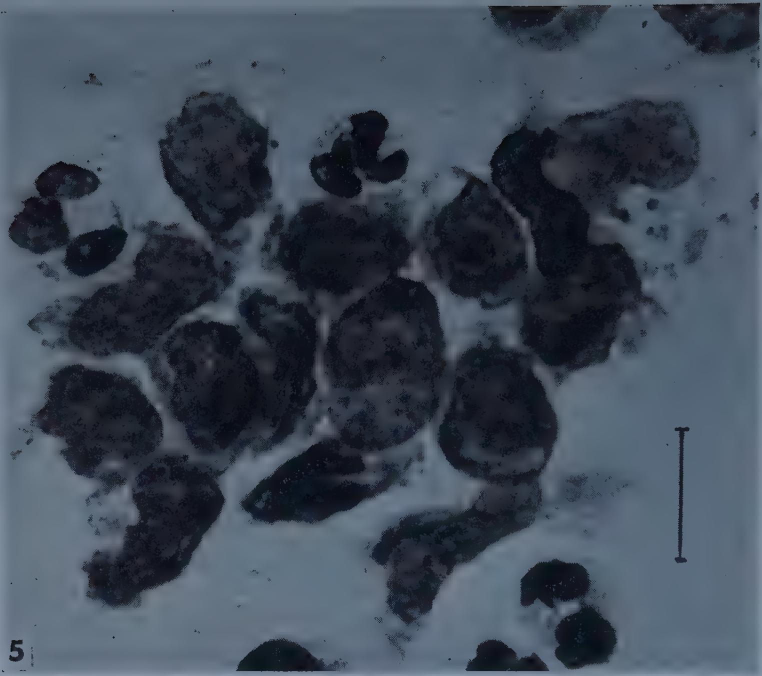

FIG. 5. 14 hrs. of inflammation. Same lesion as FIGURE 3 at higher magnification. Untreated control. Note abundance of lymphocytes and their hypertrophied forms. Scale: 10 micra. × 1800.

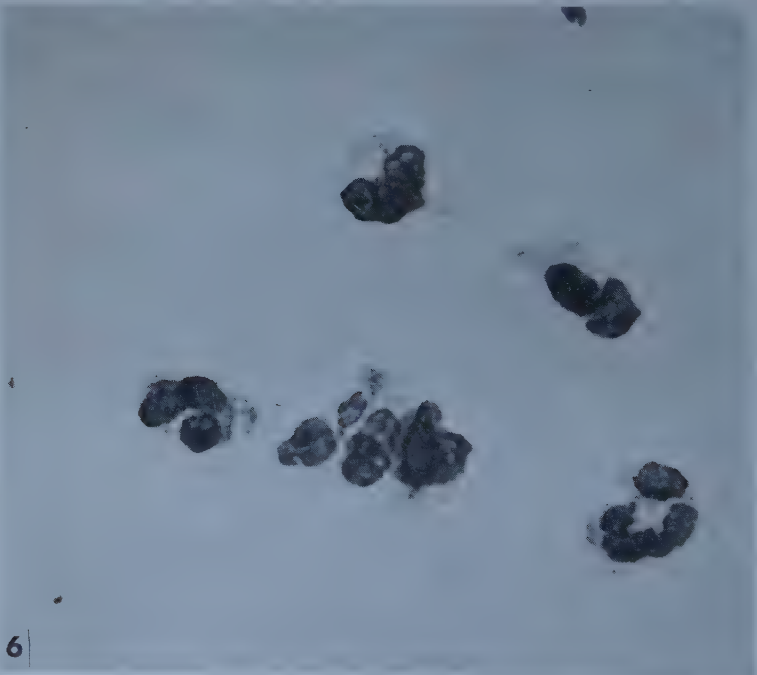

FIGURE 6. 14 hrs. of inflammation. Same lesion as FIGURE 4 at higher magnification. Previous application of topical cortisone. Note scattered neutrophils and paucity of lymphoid elements. × 1800.

well established in 3-, 5-, 7-hour cover slip preparations. At 7 hours and again at 10 hours, 0.05 cc. of the microcrystalline suspension of cortisone acetate in its aqueous vehicle was applied topically to the experimental lesion. At 10 hours, after the first application of cortisone, there was again less exudate in the experimental lesion than in the control member. The types of exudative cells in the experimental lesion differed markedly from those present in the controls at the same time. Only neutrophilic leukocytes and occasional monocytes and histiocytes were present in the cortisone-treated fields. The neutrophilic leukocytes were undergoing an unusual type of degeneration. They presented intense cytoplasmic vacuolation. At times, there was coalescence of these vacuoles, which resulted in outright hydropic degeneration of the entire cell body. There was swelling of specific granules and increased basophilia and, in some instances, complete disappearance of the granules. The nuclear lobes showed clumping and pyknosis or, less frequently, early homogenization of their chromatin. The degenerated cells were often in clumps. The occasional histiocytes presented similar, but not as pronounced, vacuolar cytoplasmic changes. Lymphocytes were almost absent. At this time, a second application of cortisone was made topically, and a small amount of India ink was touched to the lesion with a wire loop. Comparable amounts of physiological saline and India ink were applied to the control lesion.

After 12 hours of inflammation in the experimental lesion, or two hours after the second application of topical cortisone and the test for phagocytic activity, there was less over-all exudate then in the paired control. The first four columns of TABLE 1 represent the differential exudative cell counts of the experimental and control lesions of this series at this stage. Although the application of India ink had largely changed the nature of the response to a pyogenic one, continued depletion of lymphocytes, hypertrophying lymphocytes, and histiocytes after cortisone was still apparent (TABLE 1 and FIGURES 1 and 2) upon comparison with the exudate found in the control. Inhibition of phagocytic activity of the three major cell types involved (lymphocytes and hypertrophying lymphocytes as a group, histiocytes, and neutrophilic leukocytes) was of such a degree that the difference between experimental and control activity amounted in each instance to more than three times the Standard Error of the Difference (TABLE 1). In addition, FIGURE 2, which depicts a small portion of this preparation, reveals the scant phagocytic activity of the remaining neutrophils, a finding especially evident when contrasted with the portion of the control preparation (FIGURE 1) under otherwise similar circumstances. After 13.5 hours of inflammation, cellularity of the experimental exudate had recovered to the point where it was quite comparable quantitatively to the control lesions. From a qualitative standpoint, however, there was still appreciable depletion of lymphocytes and their hypertrophied forms, as well as persistence of a statistically significant depression of phagocytic activity.

In a fourth series of preparations, topical cortisone was applied to the experimental lesion at three different stages in the evolution of the inflammatory cycles: at 7, at 10, and again at 14 hours. At the same times, in the control lesion, equivalent amounts of the aqueous vehicle containing suspending agents and 1.5 per cent benzyl alcohol, but lacking in cortisone, were added topically.

Again the experimental lesion was characterized by depression of exudative cells, deletion of lymphocytes, hypertrophied lymphocytes, and the resultant histiocytes. Only a scattering of neutrophilic leukocytes and an occasional tissue histiocyte remained in the 10- and 12-hour exudative samples. After 14 hours of inflammation, 4 hours after the second topical cortisone application, the sparseness of the exudate persisted. FIGURE 4 depicts a large portion of the experimental inflammatory exudate at this stage. The fifth and sixth columns of TABLE 1 represent the differential exudative cell counts of the experimental and control lesions of this series at the 14th hour of inflammation. The infrequent lymphocytes, hypertrophied lymphocytes, and histiocytes after topical cortisone were in striking contrast to the large numbers of equivalent lymphoid elements in the adjacent control lesion. FIGURE 6 depicts the 14th hour of inflammation in this same lesion at a higher magnification. Only a few scattered neutrophils and a single lymphocyte can be made out. In FIGURE 3, in the adjacent control inflammatory exudate, after 14 hours of inflammation, the exudate shows marked cellularity. A higher magnification of a portion of this same exudate (FIGURE 5) reveals an abundance of lymphocytes and their hypertrophied forms, and only a few neutrophilic leukocytes. Due to cytoplasmic loss, the latter measure is now only 6–8 micra in diameter. Examination of the fifth and sixth columns of TABLE 1 reveals that lymphocytes, hypertrophied lymphocytes, and histiocytes made up more than half of the cell-rich control exudate, in contrast to a mere five per cent representation in the cell-poor lesion treated with topical cortisone. After 14 hours, a third application of topical cortisone in the experimental lesion and a similar amount of aqueous vehicle in the control member were coupled with small amounts of India ink to test phagocytic activity. The cover slip preparations were undisturbed for 10 more hours, and differential exudative cell counts were made at 24 hours of inflammation.

Even during these closing hours of the first day of inflammation, the customary tendency to a pyogenic exudate after application of India ink was somewhat in evidence. Such a tendency, however, in no way masked the by now typical effect of the third application of topical cortisone: diminution in exudative cell numbers; depression of lymphocytes, hypertrophied lymphocytes, and histiocytes; and, finally, inhibition of phagocytosis, both as to number of cells found to be actively phagocytic and as to number of lymphoid elements potentially available for phagocytic activity. This inferiority of the treated inflammatory exudate was maintained for another 2.5 hours, after which the experiments were completed.

The fifth and sixth series of experimental lesions were treated with topical cortisone powder suspended in homologous serum. Applications were made at 9, 11 and again at 15 hours of inflammation. The control lesion received topical homologous serum alone. In contrast to the first four series described above, topical cortisone powder suspended in homologous serum produced no observable effects in the leukocytic cycles common to both the experimental lesions and the controls in response to the initial inflammatory stimuli.*

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* Recently, Barber and Delaunay[109] have presented a reasonable explanation for this lack of activity of serum–cortisone preparations. These workers were able to obtain active serum–cortisone preparations by prior incubation of suspended cortisone crystals in serum for relatively long periods of time.

Fragmentation, partial lysis, and/or phagocytosis of degenerating lymphoid elements were not found in any of the preparations.

Discussion

ACTH and cortisone have been shown to alter the host response to a variety of infectious agents, both in experimental infection and in the course of naturally occurring disease. This alteration has been deleterious to the host resistance, with but few exceptions,[47, 48, 49, 50] in a number of species infected with many different agents. For the most part, there has been little observed difference between the effects of ACTH and cortisone on a given disease state but, in cases in which different effects have been observed,[48, 51, 52, 53] cortisone has appeared as a more potent inhibitor of resistance than ACTH. In addition to increasing the morbidity and mortality of many induced infections, cortisone treatment has resulted in the development of spontaneous infections in some species,[53, 54] and in recrudescence of chronic or latent infections.[55]

Among the bacterial infections adversely affected by ACTH or cortisone are those caused by the pneumococcus,[47, 48, 56, 57, 58, 59] staphylococcus,[47, 60] streptococcus,[59, 61, 62, 63] tubercle bacillus,[64, 65, 66, 67, 68] and the spirochetes.[69, 70] Viruses whose pathogenicity is enhanced by ACTH and cortisone administration include the poliomyelitis,[51, 52, 71] Coxsackie,[72] influenza,[73] mumps,[73] Japanese B encephalitis,[74] and vaccinia viruses.[60] In Chagas's disease, ACTH had no effect on the *Leishmania*,[75] which persisted in the blood and tissues in the face of diminished myocarditis. Fungus infections[54, 60, 76] and malaria[55, 77, 78] are also more severe if the host receives cortisone during the course of the infection.

The cellular mechanisms of the inhibitory effects of parenterally (systemically) administered ACTH or cortisone upon local inflammation have been established at four different levels: (1) in the lymphocytopoietic tissues; (2) in the peripheral blood stream; (3) in the vasculature of the local area of inflammation; and (4) in the local area of inflammation.

As mentioned in the introduction, three schools, of Metchnikoff, Maximow, and Downey, have emphasized the importance of the lymphocyte as a mobile and blood-contributed source of histiocytes or macrophages in the field of inflammation. The effects of systemically administered ACTH or cortisone upon the lymphocytic tissues and lymphocytopoiesis become, therefore, of considerable interest even to the student of local inflammation. The injurious effect of these hormones upon the lymphocytes in the lymphocytic tissues observed by Dougherty and White[79, 80] and by Simpson and her associates[81] has been well documented by the work of many recent investigators.[71, 82, 83, 84, 85] Thus, there is general agreement that the basic organ sources of the lymphocytes are subjected to injury by the hormones under study and that, consequently, the delivery of these defensive cellular units can be seriously impaired, at least *pro tempore.*

Transport of the defensive cellular units, the white blood cells, is a function of the blood. Depletion of these units in the circulating blood by systemically administered ACTH or cortisone as a consequence of impaired hematopoiesis or on the basis of as yet unestablished but more-direct effects must again produce decisive results in the local inflammatory picture. Lymphocytopenia,[86,] [87, 88] monocytopenia,[89] and eosinopenia[88] have been well established as consistently occurring phenomena following such hormonal or equivalent treatment. The one exception, the increase in circulating neutrophils reported after adrenal stimulation by adrenocorticotrophic hormone in mice and rats by Dougherty and White[86] and later confirmed for dogs[87] and man,[88] serves to point out that mere numerical ascendancy does not necessarily insure unimpaired functions of a defensive nature. Crepea, Magnin, and Seastone[90] found that neutrophilic leukocytes in blood obtained from patients under treatment with ACTH or cortisone exhibited decreased phagocytic activity for type I pneumococci. Systemic administration of the hormones under discussion or their equivalents then serves to impair transportation of the defensive cellular units, or to inhibit important defensive activities of such units while in transit.

Successful participation of the blood-contributed, defensive cellular units in local inflammation depends upon successive completion of margination, diapedesis, and migration within, through, and away from the local vasculature, in order that such defensive cellular units may reach and function in the local inflammatory site. Michael and Whorton[91] found decreased margination of leukocytes in cutaneous inflammatory reactions incited by croton oil in rabbits treated with cortisone. Spain, Molomut, and Haber[92] similarly reported delayed leukocytic margination in subcutaneous inflammation in mice. Inhibition of diapedesis of leukocytes has been implicated by Coste and his associates[93] and, more recently, by Glaser and his co-workers.[94] Impaired migration of the defensive cellular units had already been demonstrated by Heilman[95] for lymphocytes and has recently been extended to the phagocytic cells in general by Germuth, Ottinger, and Oyama.[47]

Although impairment of margination, diapedesis, and migration must impose an additional burden upon the local defensive mechanisms, the effects of ACTH and cortisone upon the vasculature itself may also act to reduce the effectiveness of the inflammatory response. With respect to this, Menkin[96, 97] has found that cortisone inhibited the increase in capillary permeability caused by leukotaxine but not by exudin. Conversely, ACTH repressed the increase in capillary permeability caused by exudin but not that caused by leukotaxine.

There remains a consideration of the cellular mechanisms of the inhibitory effects of parenterally (systemically) administered ACTH or cortisone upon inflammation at the actual local inflammatory site. Early reports[98, 99] of such inhibitory effects were expressed in broadly descriptive terms relating inhibition of the growth of granulation tissue in wounds to treatment with cortisone and ACTH. There followed work relating inhibitory mechanisms to diminished inflammatory reactions.[100, 101, 102]

Recently, attention has been centered upon the inhibition of individual defensive cellular units in such lesions and the far-reaching implications of such an impairment for local defensive mechanisms. Dougherty and Schneebeli[30] described cortisone inhibition of polymorphonuclear leukocytes in the area of localized inflammation in sensitized and adrenalectomized mice. Rebuck, Smith, and Margulis[35, 36, 37] reported inhibition of neutrophilic phagocytosis in acute experimental inflammations in patients treated with ACTH, with only rare inhibition of neutrophilic verdoperoxidase. Spain, Molomut, and Haber[92] described a necrotizing action of cortisone resulting in scant polymorphonuclears in subcutaneous inflammation in mice. Diminution of the polymorphonuclear response in inflammation by cortisone has been repeatedly affirmed.[47, 91, 94, 103, 104]

Diminished lymphocytes at the local inflammatory site after systemically administered cortisone were observed by Michael, Cummings, and Bloom.[105] Rebuck, Smith, and Margulis[34, 36, 37] depicted inhibition of lymphocyte participation in human skin windows after ACTH therapy. Similarly, hypertrophied lymphocytes were almost absent. Lymphocyte depletion of the inflammatory site has been confirmed as a result of hormonal action or its equivalent by several additional groups of workers.[55, 65, 103, 104, 106, 107, 108]

Impairment of macrophagic, histiocytic, or mononuclear activity in inflammation and tissue culture has been repeatedly observed after systemically administered ACTH, cortisone, or their equivalents.[66, 91, 92, 107, 109] Dougherty and Schneebeli,[30] Rebuck, Smith, and Margulis[35, 36, 37] and Schmidt and Squires[55] outlined one mechanism for such macrophagic inhibition as being an inhibition or obliteration of the proliferative and transformational activities involved in the production of macrophages from lymphocytes.

Similar depletion of other defensive cellular units and connective tissue elements of the reacting inflammatory site has been described for eosinophils,[35, 37, 108] mast cells,[91] and plasma cells.[106, 107, 108]

In the study of the cellular mechanisms operative in the interruption of leukocytic cycles by topical cortisone in acute inflammation in man, inhibition of the defensive cellular units at the sites of organ supply and blood transport are not pertinent.[2, 13] Whether topical cortisone effects impairment of margination, diapedesis, and migration of the defensive cellular units remains undetermined. The experiments described above point, however, to diminished neutrophilic leukocytic response, deleted lymphocytes, hypertrophied lymphocytes and diminished histiocytes after application of topical cortisone to acute inflammation in man (FIGURES 3 to 6, TABLE 1). Furthermore, phagocytic activity has been interrupted, as evidenced by decreased numbers of actively phagocytic cells among the exudative cells and by ablation of the lymphocytes and hypertrophied forms, which comprise an important, blood-contributed, mobile source of large phagocytic cells (TABLE 1, FIGURES 1 and 2).*

There remains to be accomplished an integration of these affections of the defensive cellular mechanisms in local inflammation with the great body of observations pertaining to changes in antibodies,[110, 111] local vasculature,[112] and the connective tissues proper.[113, 114]

Summary

A series of permanent fixed preparations of *in vivo* samplings of the cellular exudates were obtained from acute inflammatory lesions in man. The epithelium was scraped away from a small area of skin, after which an inflammatory excitant (diphtheria toxoid) was applied and the lesion covered with a cover slip. Within an hour the cells of the inflammatory exudate had migrated to

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* It should be kept in mind that deficiencies of ACTH, cortisone, or their equivalents produce comparable impairment of phagocytic activity,[115 116 117] and that replacement therapy has been found to actually enhance phagocytic activity.[116 117]

the under surface of the cover slip. When such migration had been effected, the cover slip was removed and additional cover slips were placed over the same lesion at timed intervals. The preparations so obtained were air-dried and stained like blood smears.

Eleven control series were obtained from lesions prepared in untreated volunteers. Six experimental series were obtained together with paired controls in human volunteers with experimental lesions to which topical cortisone was subsequently applied. The time at which the epithelium was removed marked the time inflammation began. In fifteen controls, the cellular cycle of acute inflammation consisted of early migration of neutrophilic leukocytes with minor participation by monocytes and tissue histiocytes. At 8 to 12 hours, lymphocytes were present in increasing numbers. At 12 to 14 hours, there was neutrophilic degeneration and contrasting lymphocytic hypertrophy. From 14 to 24 hours, lymphocytic hypertrophy eventuated in lymphocytogenous (and histogenous) histiocytic predominance. Subsequent to topical application of cortisone, there was marked depression of lymphocytic participation at the 8–12-hour period of inflammation and, as a consequence of the hypertrophying lymphocytes, at 12 to 14 hours. Retardation and incompleteness of the cellular sequences were paralleled by depression of phagocytosis, which was effected both by inhibition of phagocytic ability of neutrophilic leukocytes as well as by depression of cells phagocytic or potentially so, *i.e.*, lymphocytes, hypertrophying lymphocytes, and resultant histiocytes.

The literature was reviewed with reference to impairment of defensive cellular units by ACTH, cortisone, or their equivalents at four graded levels, any one of which, if affected, could in turn modify local inflammatory responses (1) at the level of hematopoiesis, particularly lymphocytopoiesis; (2) during transport in the blood; (3) at the time of migration from the local vasculature and finally; (4) at the local inflammatory site.

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INHIBITION OF THE PHENOMENON OF LOCAL TISSUE REACTIVITY BY CORTICOSTEROIDS, SALICYLATES, AND COMPOUNDS RELATED TO SALICYLATES*

By Gregory Shwartzman and S. Stanley Schneierson
Department of Microbiology, The Mount Sinai Hospital, New York, N. Y.

The phenomenon of local tissue reactivity is a profound vascular damage and necrobiosis elicited by combined local preparatory and intravenous provocative injections of certain bacterial filtrates or combined local preparatory injection of bacterial filtrates and intravenous provocative injection of antigen antibody complexes or certain colloidal agents. The reaction can only be elicited when the provocative injection is made into the general circulation. No satisfactory explanation is yet available concerning the necessity of introducing the provocative agent into the vascular system. The following long list of substances tested failed to modify the phenomenon; namely, acetylcholine, adenosine-5-phosphoric acid, alypin, antiplatelet serum, amino acids in various combinations, ascorbic acid, atropine, benadryl, biotin, calcium chloride, calcium gluconate, casein hydrolysate, choline, cocaine, congo red, curare, DCA, dicumarol, distilled water, estradiol, ether general anesthesia, folic acid, glucose, heparin, hesperidin, histaminase, histamine, india ink, inositol, lemon "citrin," milk, Niagara sky blue, nicotinic acid, pantothenic acid, paraminobenzoic acid, physostigmine hydrochloride, pilocarpine, progesterone, pyribenzamine, pyridine in doses reducing significantly the blood platelet count, pyridoxal, pyridoxamine, pyridoxine, rat-liver extract, rat-spleen extract, riboflavin, sodium oxalate, sulfadiazine, sulfanilamide, sulfathiazole, thiamine, α-tocopherol, toluidine blue, trypan blue, trypsin, urethane, vitamin K water-soluble and oil-soluble preparations, wheat-germ oil, yeast extract, BAL, mapharsen, partial exsanguination, thyroidectomy, and anthisan. The negative results obtained with the substances just mentioned seem to exclude several possible mechanisms; namely, alteration of blood coagulation, release of histamine, and modification of the response of the peripheral and central nervous systems.[1] There are, however, a few substances that can effectively suppress the phenomenon. As shown by Becker,[2] and corroborated by Schlang[3] and by Stetson and Good,[4] nitrogen mustard, benzol, and x-ray irradiation are capable of inhibiting the phenomenon. Becker postulated that these agents produce the suppression by rendering the vascular endothelium of the prepared skin site nonreactive, thus preventing it from reacting to the provocative agent. In studying the mechanism of this inhibition, Schlang and Stetson and Good found that protection of the prepared site from the effect of nitrogen mustard failed to influence the inhibition of the reaction. In contrast, protection of the lower limbs from the action of nitrogen mustard by aortic occlusion decreased or prevented the inhibition. They concluded justly that the effect of nitrogen mustard was the result of systemic rather than local action. We found that ACTH and cortisone in large doses administered at a suitable time may suppress

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the phenomenon.[5, 6, 7] The experiments with ACTH are recorded in TABLE 1. As may be seen from this table, ACTH did not influence the phenomenon when injected prior to the preparatory injection. The administration of a sufficient dose of the hormone (12.5 mg. per animal) preceding the provocative injection completely suppressed the phenomenon in 8 out of 10 animals tested, however. Smaller doses of ACTH similarly injected produced only irregular inhibition. The results may be considered highly significant if contrasted with the high incidence of reactions obtained in the control groups. It is also obvious that the suppression of the phenomenon could not be attributed to the presence as contaminants of oxytocic and pressor substances in the ACTH solutions, since surgical pituitrin containing larger amounts of these substances than those present in ACTH solutions failed to produce the effect. In later studies, it was found that the optimum time for obtaining inhibition of the phenomenon with ACTH injected intramuscularly or intravenously was about two hours preceding the provocative injection of the toxin. It was of interest that ACTH was capable of protecting the tissues from damage by agents to which they were sensitive without removing the state of sensitization. This effect is similar to clinical therapeutic results, in which ACTH suppresses manifestations of diseases without removing their cause. The experiments just described were corroborated by Marcus and Donaldson[8] and Storck.[9] These observations seem to relate, in some way, the provocation of the phenomenon to adrenocortical function.

TABLE 1

INHIBITION OF THE PHENOMENON OF LOCAL TISSUE REACTIVITY BY ACTH

Preparatory treatment			Preprovocative treatment			Reactions		
Substance	Dose	Hr. prior to I.D. inj.† of bacterial filtrate	Substance*	Dose	Hr. prior to I.V. inj.‡ of bacterial filtrate	Strongly positive	Doubtful	Negative
—	—	—	—	—	—	26	0	2
—	—	—	PS	0.5 oxytocic u. + 0.5 pressor u.	2	4	0	1
—	—	—	PS	0.75 oxytocic u. + 0.75 pressor u.	1	5	0	0
—	—	—	PS	0.75 oxytocic u. + 0.75 pressor u.	2	6	0	0
ACTH	2 × 5 mg.	18 and 1	—	—	—	4	0	0
ACTH	12.5 mg.	2	—	—	—	6	0	0
—	—	—	ACTH	2.5 mg.	2	1	0	1
—	—	—	ACTH	5 mg.	2	2	3	1
—	—	—	ACTH	2 × 5 mg.	18 and 1	1	2	1
—	—	—	ACTH	12.5 mg.	2	2	0	8

* PS = Pituitrin-S, Parke Davis & Co., 1 ml containing 20 oxytocic units and 20 pressor units. ACTH = Adrenocorticotropic hormone, batch H3706, 12.5 mg containing 0.5 oxytocic units and 0.375 pressor units.

† I.D. inj. = Intradermal injection.

‡ I.V. inj. = Intravenous injection.

About the same time we observed the effect of ACTH, Smith and Humphrey[10] produced inhibition of the phenomenon with sodium salicylate. In the light of the action of ACTH and cortisone, the mechanism of the effect of sodium salicylate was interpreted by us as being possibly dependent on adrenocortical function. In extending the observations, a number of other compounds were tested from this point of view. The results are recorded in TABLE 2.

TABLE 2

INHIBITION OF THE PHENOMENON WITH SALICYLATES AND DERIVATIVES

50 per cent inhibition	*No inhibition*
Sodium salicylate	2:4-Dihydroxybenzoic acid
Acetyl salicylic acid	2:5-Dihydroxybenzoic acid
3-Hydroxy-2-phenycinchoninic acid	4-Methoxy-2-hydroxybenzoic acid
	5-Methoxy-2-hydroxybenzoic acid
	Phenyl salicylate
	Acetanilide
	Aminopyrine
	Paraminosalicylic acid
	Paraminobenzoic acid
	Gentisic acid

TABLE 3

Treatment			Results		
Substance	Total dose, g./kg.	Method of administration	Pos.	Doubtful	Neg.
Sodium salicylate	1.6	I.P. 20 min. prior prep. inj. 18 h., ½ h. prior prov. inj. and 2 h. after prov. inj.	12	0	17
Calcium pantothenate	0.8	*idem*	6	0	0
Sodium salicylate and calcium pantothenate	1.6 0.8	*idem*	0	1	5
Sodium salicylate and calcium pantothenate	1.6 0.4	*idem*	0	0	10
Sodium salicylate and calcium pantothenate	1.6 0.2	*idem*	2	0	4
Controls			65	3	3

It is of interest that the three compounds sodium salicylate, acetyl salicylic acid and 3-hydroxy-2-phenycinchoninic acid* had one feature in common, namely, ability to stimulate the function of the adrenal cortex.

As recorded in TABLE 2, the inhibition of the phenomenon was usually obtained in only about 50 per cent of the rabbits tested. In interpreting these results, it occurred to us that the variability may be due to the individual differences in the adrenocortical response to the stimulation. Our attention turned then to calcium pantothenate, since it is now assumed that this substance plays an important role in the synthesis of steroids by the adrenal cortex. It was of interest, therefore, to determine the effect of treatment with the above-mentioned three substances in combination with calcium pantothenate.

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As may be seen from TABLE 3, a massive dose of sodium salicylate suppressed the phenomenon in 17 out of 28 rabbits. Calcium pantothenate alone in a large dose failed to produce any effect. Administration of sodium salicylate in combination with calcium pantothenate, however, resulted in complete inhibition of the phenomenon in all animals tested. Thus, calcium pantothenate enhanced the suppressing effect of sodium salicylate without itself affecting the phenomenon.

The administration of calcium pantothenate in combination with acetyl salicylic acid or 3-hydroxy-2-phenycinchoninic acid also increased the protection against the phenomenon from 50 to 100 per cent. The complementary role of calcium pantothenate is of interest. It is suggested that in time of stress the adrenal cortex may not be able to produce an adequate quantity of hormones, not because of its limited functional ability, but through lack of a sufficient amount of "building materials" in a normal economy. The tentative assumption is made that by making an ample supply of calcium pantothenate available the adrenal cortex, when properly stimulated, may then be able to meet the emergency requirements for rapid synthesis and an unusually large output.*

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* The following compounds, 2:4-dihydroxybenzoic acid, 2:5-dihydroxybenzoic acid, 4-methoxy-2-hydroxybenzoic acid, and 5-methoxy-2-hydroxybenzoic acid, were synthesized by Dr. J. Lowenthal of the University of Saskatchewan and tested at his suggestion.

EFFECT OF CERTAIN CORTICOSTEROIDS AND OF GROWTH HORMONE OF NUCLEOPROTEINS OF LYMPH NODES

By Edward H. Kass, Mary I. Kendrick, and Maxwell Finland[*]
Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard); Boston City Hospital; and Department of Medicine, Harvard Medical School, Boston, Massachusetts

One of the most striking and consistent anatomic changes that follow the administration of adrenocorticotropic hormone or cortisone to experimental animals is atrophy of lymphoid tissue. Because such tissue represents a large repository of nucleic acids in the body, the effect of adrenocortical steroids on the nucleic acid content of lymph nodes was investigated. The popliteal lymph nodes of rabbits are discrete and easily obtained, and were, therefore, used as the test system.

The effects of various steroids and other hormones on the wet weights of carefully trimmed popliteal lymph nodes of male rabbits weighing 2.5–3.5 kg. are shown in FIGURE 1. All hormones were administered intramuscularly or subcutaneously in five daily doses and the nodes were obtained 24 hours after the last injection. The mean weight of normal popliteal nodes is 178 mg., and twice the standard deviation of the mean of control lymph nodes is charted. Cortisone, in the usual suspending medium, or as a crude saline suspension, led to significant atrophy of the nodes. Purified corticotropin obtained from Drs. E. B. Astwood and M. S. Raben was administered in oil and beeswax, and this preparation also caused atrophy of the nodes. A long-acting Armour preparation of ACTH was not effective in inducing atrophy and, instead, seemed to induce hyperplastic changes. Recent experiments suggest that this may have been a rebound effect due to insufficient dosage of ACTH and that larger doses or more frequent administration of the gel suspension of ACTH may induce atrophy of the nodes. The administration of hydrocortisone induced lymphoid atrophy. The free alcohol was more effective in this respect than the acetate, presumably because of slow hydrolysis of the latter. Corticosterone caused the nodes to become hyperplastic, and growth hormone alone induced mild and probably insignificant hyperplasia. Growth hormone given in combination with either cortisone or hydrocortisone did not reverse the effect of the steroids and, when given with corticosterone, overcame the effect of that steroid in inducing mild hyperplasia.

Homogenates of the lymph nodes were analyzed for contents of desoxypentose and pentose nucleic acids by the methods of Schmidt and Thannhauser and of Schneider. Only phosphorus values are given on the charts but, in most instances, duplicate analyses for the carbohydrate content of the nucleic acids were also conducted, with good general agreement between the carbohydrate and phosphorus analyses. Although the data, as presented, relate to the wet weights of the lymph nodes, analyses of the nitrogen content of the homogenates showed a relatively fixed ratio of wet weight to nitrogen.

Cortisone (FIGURE 2) has no significant effect on the concentration of DNA, despite the marked lysis of lymphocytes and the disintegration of nuclei that occurs in the nodes, but the PNA concentrations in nodes were distinctly reduced.[1] Crude saline suspensions of cortisone and highly purified ACTH acted in the same mamer. The less-active gel suspensions of ACTH did not reduce the concentrations of PNA, whereas hydrocortisone as the acetate or free alcohol did so. Corticosterone failed to reduce PNA concentrations in the node, just as it failed to reduce the weights of the nodes. There was no evidence that growth hormone overcame the effects of cortisone or hydrocortisone in reducing PNA.

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Some of the possible implications of these experiments may be noted. First, despite the findings of Bush that the adrenal cortex of the rabbit secretes chiefly if not entirely, corticosterone,[2] ACTH induced changes in nodal weight and PNA concentrations that could not be reproduced by corticosterone. These changes did occur, however, after injection of the same amounts of cortisone or hydrocortisone. One possible explanation for this discrepancy is that corticosterone is quantitatively less effective than the other two hormones in its effect on lymph nodes, and that ACTH simply causes the rabbit adrenal gland to secrete huge amounts of corticosterone. This seems unlikely, in view of the relatively large amount of corticosterone used in these experiments (10 mg. per day), although it is not clear how much of the injected hormone became soluble and available to the animal tissues. An alternative explanation is that, under the influence of large doses of ACTH, the rabbit adrenal may produce hormones more closely akin to cortisone and hydrocortisone. A third possibility is that the effects of the steroids used are conditioned by the presence of other adrenocortical hormones or by metabolic products of the hormones, which may potentiate the action of endogenously secreted corticosterone.

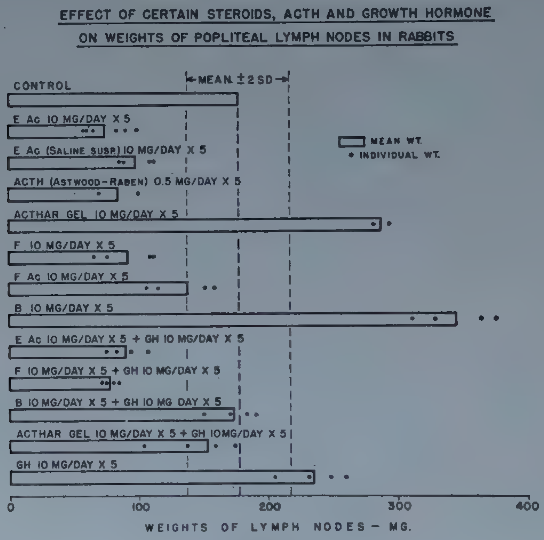

FIGURE 1

A second possible implication of these observations is that the effects on lymph nodes of the steroids that were studied may parallel their antiarthritic effect in man, inasmuch as corticosterone is ineffective as an antiarthritic agent, whereas ACTH, cortisone, and hydrocortisone are active in this regard.[3]

A third deduction that may be made from these studies is that the inhibition of antibody formation by cortisone is due, at least in part, to its effect on the PNA in the nodes.[1] When antigens are injected into a tissue depot of a rabbit not receiving cortisone, the regional lymph nodes become hyperplastic, and antibody may appear in them within two to three days. The production of antibody in the regional nodes is accompanied by the appearance of increased numbers of large immature cells with basophilic cytoplasm. The basophilia of the cytoplasm of these cells has been shown by several workers to be due to the presence of PNA, the nuclei of these and other cells being composed largely of DNA.[4, 5] During antibody formation, cells with high contents of PNA become more numerous, and the concentration of PNA in lymph nodes increases. Because of the effect of cortisone on PNA concentrations of normal lymph nodes, and because cortisone inhibits antibody formation, it seemed possible that cortisone might interfere with PNA metabolism and thus with antibody formation.

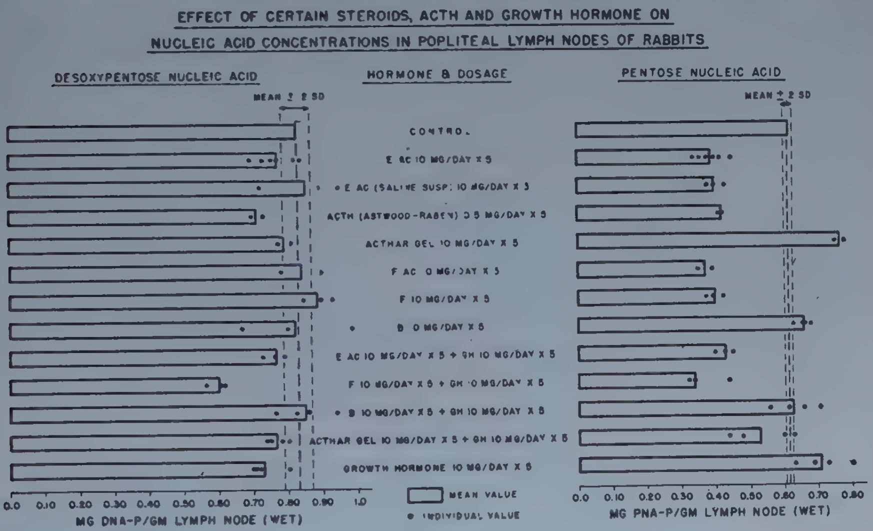

FIGURE 2

To test the hypothesis that cortisone may inhibit antibody formation in lymphoid tissue through its effect on PNA, sterile suspensions of a rabbit's own erythrocytes were injected into one footpad and equal numbers of foreign erythrocytes (sheep or horse) were injected into the contralateral paw. The injection of heterologous antigenic cells was followed by a rapid and regular increase in the weight of the node (FIGURE 3), with accompanying increases in the concentration of PNA. On the side receiving nonantigenic autologous cells, hyperplastic changes and changes in PNA did not occur. Cortisone caused the nodes to be smaller than the corresponding nodes of animals not receiving hormone, but the weight of nodes in cortisone-treated animals on the side receiving foreign cells was always greater than on the side receiving autologous cells. Similarly, the PNA concentrations in the cortisone-treated animals were higher in those nodes receiving antigenic cells than on the side receiving the autologous erythrocytes but, in all instances, cortisone depressed the PNA values below those found in control animals receiving erythrocytes but not cortisone. The concentrations of DNA were not greatly affected by the administration of cortisone, although antigenic stimulation led to late increases in DNA. Antibody formation was diminished to varying degrees, both in serums and lymph-node homogenates of cortisone-treated animals, as compared with the controls.

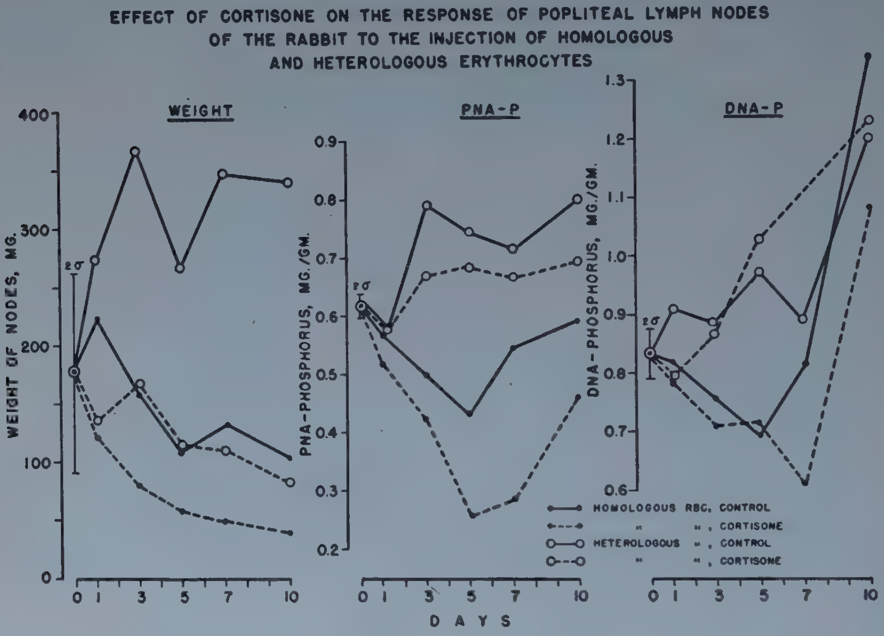

FIGURE 3

The data suggest that in the rabbit cortisone may alter PNA metabolism in lymph nodes in some manner, and that the response of lymph nodes to stimulation with homologous and heterologous erythrocytes is altered by the hormone. Preliminary experiments indicate that hydrocortisone has a similar effect.

The detailed histologic and histochemical data will not be presented here, but they can be summarized by the statement that cortisone diminishes the number of large PNA-rich cells in the antigenically stimulated nodes, and brings about significant increases in the numbers of macrophages in the nodal sinuses.

An interesting by-product of this investigation was the observation that, when erythrocytes were injected into normal rabbits, they were promptly found in the nodal macrophages, where they persisted for one to two days and then disappeared. In the cortisone-treated animals, however, the erythrocytes were phagocytosed in large numbers but remained visible for as long as 7–10 days. In both the control and the cortisone-treated animals, autologous cells persisted longer than the heterologous cells. The observations suggest that one action of cortisone on the reticuloendothelial system is to inhibit the capacity of the phagocytic cells to dispose of phagocytosed material.[6] Whether alterations in nucleic acid metabolism are related to this phenomenon remains to be determined, but the observations suggest another means whereby the function of the reticuloendothelial system is disturbed by the action of cortisone.

Similar observations were made recently with a protein antigen. When proteins are labeled with radioactive iodine, and injected subcutaneously into the paws of rabbits, the rate at which nonprotein iodine accumulates in the urine is a measure of the rate of mobilization and hydrolysis of the injected substance from the tissue depot, the thyroidal uptake of the radioactive iodine being minimized by the prior administration of iodides to the animals. Under such circumstances, cortisone causes both native and foreign proteins to be mobilized more slowly from the tissue sites of deposition although, as in the case of erythrocytes, the effect on disposition of the homologous protein is greater than the effect on the foreign protein.

In summary, cortisone, hydrocortisone, and ACTH induce changes in morphology and chemical composition of lymph nodes of the rabbit. Cortisone decreases the PNA content of nodes, inhibits antibody formation, and decreases the rise in PNA that accompanies antibody formation. Evidence is presented to suggest that cortisone also affects the capacity of reticuloendothelial and perhaps other cells to dispose of foreign and native substances that have been deposited in the tissues.

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CORTISONE AND IMMUNITY TO HOMOIOGENEOUS TISSUE—LOSS OF "INDIVIDUALITY DIFFERENTIALS" FROM TISSUES OF CORTISONE-TREATED RATS

By Herbert C. Stoerk
Merck Institute for Therapeutic Research, Rahway, N. J.

The group of diseases that is alleviated by the administration of cortisone is generally considered to be of allergic etiology. Thus far, the immunological basis of these allergic conditions remains undisclosed, and it is therefore impossible to decide whether or not cortisone depresses immunity to such unknown antigens. None of the "collagen diseases" have found their exact counterpart in sensitized experimental animals, but it appears from such experiments that certain manifestations of the "delayed" rather than those of the "immediate" type of hyperreactivity resemble more closely lesions in these human diseases. In previous studies,[1, 2] it was observed that the delayed (tuberculin) type of hyperreactivity was strikingly suppressed when cortisone was administered several days before the reactions were elicited. Under the same treatment (at the dose level of cortisone employed), manifestations of the immediate type of hypersensitivity (phenomenon of Arthus, anaphylaxis) could be provoked with undiminished intensity, indicating that no general loss of reactivity of tissues or blood vessels had occurred. The failure of a short treatment with cortisone to suppress established (immediate) reactivity due to circulating antibody of the classic type appeared quite expected. Although the administration of cortisone was shown to inhibit the formation of this type of immune body,[3, 4] it had no action upon its degradation,[5] so that, following a short course of treatment, the loss of antibody is far from complete and sufficient antibody remains to react with the specific antigen.

In the delayed (tuberculin) type of hyperreactivity, a different situation obtains. This form of hyperreactivity was shown by Landsteiner and Chase[6] to be transferable only by viable lymphoid cells and not by injured cells or serum. Since the immune principle involved, at least as far as it is available for circulation, is directly dependent upon the integrity of lymphoid cells, one may expect *a priori* that the well-known destructive action of cortisone upon these cells should be associated with a diminished reactivity.

An immune principle sessile in viable lymphoid cells, strikingly resembling that of Landsteiner and Chase, was observed by Kidd and Toolan[7] to be associated with acquired resistance to grafting with tumor cells. The finding that this type of immunity also is diminished when lymphoid tissue is suppressed by the administration of cortisone,[8] points to another similarity between these two forms of immunity associated with "sensitized" cells. From circumstantial evidence it has been suggested in the past that the success of homoiotransplants of neoplastic tissue is largely dependent upon the same factors that determine the compatibility of homoiografts of normal tissue. Quite in keeping with this idea was the finding by Medawar *et al.* that homologous skin grafts persisted several times longer in cortisone-treated rabbits than in untreated controls.[9]

Immunity against antigens that in some way seem specific for each individual of a given species has long been postulated to account for the incompatibility of homoiografts. These undefined antigens were observed to incite a rather characteristic lymphocytic response and have been termed by L. Loeb[10] "individuality differentials" or "homoiotoxins."

The experiments summarized in the following deal with the sensitization of rats with homoiogeneous normal tissues and with the effect of cortisone upon this "immune system."

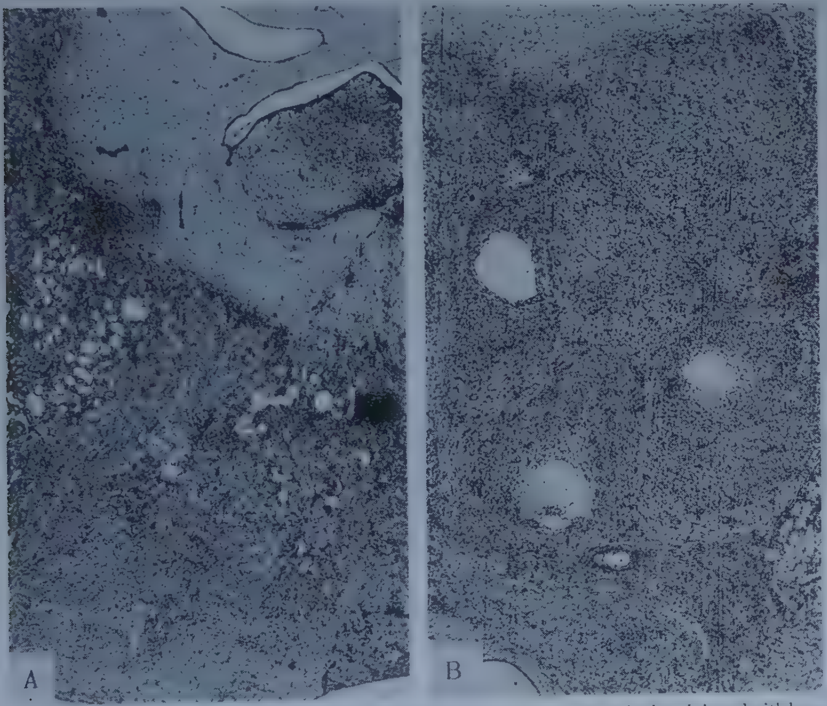

FIGURE 1. (A) Unsuccessful homiograft of ovarian tissue in kidney of ovariectomized rat injected with homologous spleen (respresentative of 9 of 12 grafts that failed to take). (B) Functional ovarian tissue graft with corpora lutea, growing follicles, and ova in uninjected ovariectomized rat (11 of 14 grafts were successful).

Previously it was observed[11] that a single injection of viable normal lymphoid cells regularly produced resistance to grafting with 250,000 lymphosarcoma cells. The resistance observed appeared after a delay of several days and persisted for about four weeks. It was limited to the number of tumor cells in the inoculum and was not detectable when 2,500,000 lymphosarcoma cells or more were used for challenge. It was also seen that the implanted tumor cells failed to grow only when viable, homologous lymphoid cells were injected previous to the inoculation. Homologous cells injured by freezing or moderate heating (45°C) caused no depression of growth of the tumor implants. Viable autologous or heterologous lymphoid cells were likewise without effect. Lymphoid cells of single rats were capable of immunizing the majority of unselected groups of other rats, and no differences in blood groups were discernible in the stock of rats employed (Sprague-Dawley). The development of the resistance was prevented by the administration of cortisone.

From the experiments summarized below, the possibility appears excluded that the observed immunity against lymphosarcoma was directed against a specific type of tissue (lymphocyte-lymphosarcoma cell). Under ordinary circumstances, homoiogeneous transplants of endocrine organs like those of other normal tissues fail to persist. Homologous endocrine organs can, however, be transplanted with success to animals in which the endocrine organs corresponding to the implanted tissue have been previously removed.[10] The reason for this is not clearly recognized, but it appears likely that pituitary hormones and other trophic influences may represent a growth stimulant strong enough to overcome the incompatibility of such homoiografts.

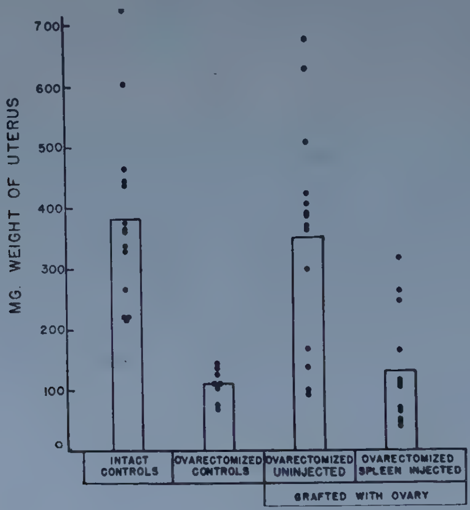

FIGURE 2. Weight of uterus in ovariectomized rats injected with viable, homologous, splenic cells one week before grafting with homologous ovary. The rats were killed and their uteruses were weighed 10 weeks after small fragments of ovarian tissue were implanted into each animal's left kidney.

TABLE 1

ACQUIRED RESISTANCE TO GRAFTING WITH LYMPHOSARCOMA FOLLOWING INJECTIONS WITH HOMOLOGOUS TISSUES*

Tissues injected (3 times)	Tumor volume, cu.cm.		No. of takes
	Ave.	Range	
Spleen intact	0	—	0/14
Kidney intact	4.4	0–43.5	1/9
Kidney frozen	48.3	10.6–56.1	5/5
Liver intact	0.2	0– 2.0	1/8
Liver frozen	46.5	9.8–74.9	10/10
RBC intact	26.1	0–59.6	7/10
(Noninjected)	44.1	0–83.9	19/20

* Three intravenous injections on alternate days of the various cell suspensions (20% in saline) were given to each of the groups except the controls. Seven days after the last injection all rats were inoculated with 250,000 lymphosarcoma cells. Tumor volume and number of takes were recorded at the 14th day after the inoculation.

The findings summarized in FIGURES 1 and 2 illustrate that, following a single injection of homologous lymphoid cells, ovarian grafts failed to persist in ovariectomized rats, while in noninjected controls the homoiografts persisted and proved functional. It appears, therefore, that homologous spleen immunizes against homologous ovarian tissue in the same manner as against homologous lymphosarcoma.

Single injections of a variety of cell suspensions other than that from lymphoid organs did not produce measurable resistance to grafting with 250,000 lymphosarcoma cells.[12] However, it is seen from the experiments summarized in TABLE 1 that repeated intraperitoneal injections of minced liver or kidney prevented takes of homologous grafts of neoplastic tissue. This effect was not observed when the parenchymal cells were injected after being subjected to freezing and thawing. These findings further indicate that organ specific antigens are not a determining factor in immunity to viable, homologous cells. It appears much rather that acquired resistance to grafting with homologous tissue is related to one and the same antigenic component, which is present in various viable tissues of the same animal but differs from one rat to another, thereby characterizing the animals biological individuality.

TABLE 2

FAILURE OF LIVER OR KIDNEY FROM CORTISONE-TREATED RATS TO PRODUCE RESISTANCE TO GRAFTING*

Tissues injected (3 times)	Tumor volume, cu.cm.		No. of takes
	Ave.	Range	
(A) Liver (control)	1.6	0–23.2	2/14
Liver (cortisone)	22.4	2.0–44.0	11/13
Kidney (control)	5.3	43.5	2/14
Kidney (cortisone)	18.3	0–64.0	10/16
Spleen (control)	0	—	0/10
Spleen (cortisone)	6.0	0–39.1	2/12
(B) Liver (control)	0	0	0/10
Liver (N-mstd.)	18.0	0–73.5	4/8
Liver (x-ray)	25.3	0–35.6	8/10
None	30.6	0–79.3	19/20

* The procedure was the same as that in the experiments summarized in TABLE 1. In group (A), organs of normal as well as of cortisone-injected rats were used for immunization. The donor rats were injected with 5 mg. cortisone daily for 5 days. In group (B), minced livers from-radiated (700 γ) and of N-mustard (4 mg./kg.) injected animals served for immunization.

From the experiments summarized in TABLE 2, it can be seen that these "individuality differentials" appear greatly diminished when lymphoid cells are destroyed by the action of cortisone or by other lymphocytolytic agents which are known to inhibit antibody formation. This finding is compatible with the interesting hypothesis ("self marker concept") of Burnet and Fenner,[13] who suggested that certain isoantigens contained in erythrocytes or lymphocytes may represent a prototype of antigen that determines a "master pattern" in antibody formation.

In the serum of animals, grafted with homoiogeneous tissue, measurable immune activity to normal, homologous tissues has been carefully searched for but has escaped detection.[14] From the experiments in FIGURE 3, it can be seen that repeated injections with viable, homologous cells "sensitized" the lymphoid cells of the recipient animals. The immune activity of these cells could be measured in a semi-quantitative fashion by their "cytotoxic" action upon homologous tumor cells. Rats in which transplants of homologous lymphosarcoma had regressed exhibited circulating antibody of an atypical type.[15] No immune activity of serum could be demonstrated in rats intensely immunized with normal, homologous cells. Like in the case of the "sensitized cells" in tuberculin hyperreactivity, nonviable lymphoid cells (freezing or heating to 45°C) exhibited no immune activity.

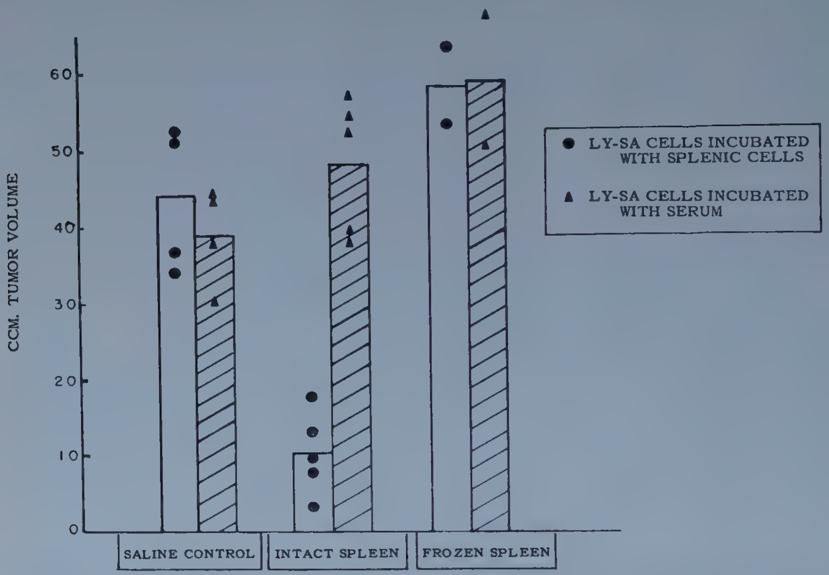
SENSITIZATION OF SPLENIC CELLS OF RATS INJECTED WITH INTACT HOMOLOGOUS SPLEEN

FIGURE 3. Three groups of 8–15 rats each were injected three times weekly, one with suspensions of intact, homologous, splenic cells, the other with an identical suspension subjected to freezing and thawing, and the third with saline. One week after the last injection, pools of serum and of saline-suspended lymphoid cells (spleen) from those three groups of rats were tested for their "cytotoxic" action upon lymphosarcoma cells. 250,000 lymphosarcoma cells were mixed with the respective material, incubated at 37°C for one hour, and then injected into groups of six susceptible rats. Each point on the graph represents the average tumor volume attained 14 days after the inoculation.

Since cells of the type as those just shown to exert immune activity against homologous tissue are known to accumulate about implants of homologous tissue,[10] it appears likely that the failure of homoiotransplants to persist is due to the immune activity of these sensitized cells. It is also likely that the destruction of these cells by cortisone explains the diminished incompatibility of homoiografts in cortisone-treated animals.[8, 9, 11]

From the experimental evidence presented it appears that, in relation to the immunity against homoiogeneous tissues, the lymphoid cells play a dual role. "Homoioantigenicity" as well as the immunity directed against it appeared to depend upon the integrity of these cells. Cortisone, which actively destroys lymphoid tissue, diminishes "homoioantigenicity" as well as established resistance to grafting with homologous tissue.

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SOME OBSERVATIONS ON MECHANISMS OF CORTICOSTEROID ACTION ON INFLAMMATION AND IMMUNOLOGIC PROCESSES*

By Thomas F. Dougherty
Department of Anatomy, University of Utah College of Medicine, Salt Lake City, Utah

A considerable body of knowledge concerning the ways adrenocortical secretions affect certain processes such as lymphopoiesis, antibody synthesis and the inflammatory response has now been accumulated. There is, however, a paucity of factual information relating to the more fundamental mechanisms by which these hormones influence the biochemical processes concerned in each of these phenomena. It seems that, at present, we are in the descriptive stage of analysis of adrenocortical hormone action and that, before we can enter with confidence the field of more fundamental investigation, the biological events which are influenced by adrenocortical hormones must be clearly delineated and methods for their accurate measurement must be designed. The following review is an attempt to present certain concepts which may serve to form a basis for further investigation of the more fundamental mechanisms of adrenocortical hormone action.

Since the reviewer has compared the anti-inflammation-producing effects of the adrenocortical hormones with the capacities of these substances to inhibit antibody synthesis and reduce resistance to infection elsewhere,[1] a review of these problems is not included here.

The Relation of the Influences of Adrenocortical Hormones on Lymphatic Tissue Growth and Synthesis of Antibody

Hormonal regulation of lymphatic tissue mass. It is well established that gonadal and adrenocortical hormones exert a moderating influence on lymphatic tissue mass.[2] There is no evidence, however, that there are any endogenously produced steroid hormones which tend to increase the amount of lymphatic tissue, although recent experiments have shown that analogues of some of the adrenocortical hormones may induce a slight increase in the weights of certain lymphatic organs.[2, 3] Whether these compounds are secreted, either normally or abnormally, by the adrenal cortex and whether they have direct or indirect growth promoting effects on lymphatic tissue is not known as yet.

The phenomenon of acute involution of lymphatic tissue differs in certain respects from that of the age involution of this tissue, although both are influenced by gonadal and adrenocortical hormones.[2] Acute involution of lymphatic tissue is characterized by a sudden loss of weight and nitrogen content followed by a rapid reconstitution to levels characteristically found in animals of the same age and sex. Age involution, on the other hand, is characterized by a gradual decline in the amount of lymphatic tissue from the time of puberty

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* Investigations from the author's laboratory reported here were financed by grants in aid from the Committee on Growth of the National Research Council acting for the American Cancer Society and from the Research Division of Armour and Company.

Generous supplies of cortisone and Hydrocortisone were provided by Augustus Gibson, M. D. of Merck and Co. Other steroid hormones were supplied by Dr. Abraham White of Chemical Specialties, and ACTH used in these investigations was received from Dr. E. Hays of Armour and Co.

through adult life and into senility. Acute involution occurs in response to a host of exogenous and endogenous pathological and physiological adaptive stimuli. The nature of these stimuli and the details of this lymphatic tissue response have been discussed more thoroughly in other publications.[2, 4] It will suffice for our purposes here to state that, with the exception of various types of irradiations, chloroethyl vesicants and pyridoxin deficiency, there are no other known noxious or adaptive stimuli capable of inducing acute lymphatic tissue involution in the absence of the adrenal cortex.[2] It is significant that gonadal hormones, unlike adrenocortical steroids, do not mediate acute involution due to stressors, although they are capable of producing acute involution when administered to intact or adrenalectomized animals.[2]

The quantitative interrelationship of hormone concentration and lymphatic tissue mass. Ablation of gonads and/or adrenocortices is followed by an increase in the mass of lymphatic tissue.[2] The degree of this increase, however, is not great and the lymphatic tissue mass of gonadectomized or adrenalectomized animals does not exceed the largest amount found in well nourished, intact animals of the same age and sex.[5] Thus, it is suggested that since there is no progressive increase or true hyperplasia of lymphatic tissue in adrenalectomized and/or gonadectomized animals, that adrenocortical and gonadal hormones act on lymphatic tissue solely by moderating or counterbalancing those forces which tend to increase lymphatic tissue.

In the absence of the adrenal cortex, stressors tend to increase rather than decrease the amount of lymphatic tissue.[6] Thus, subjection of an adrenalectomized animal to stressful stimuli results in a true hyperplasia of lymphatic tissue; *i.e.*, the lymphatic organs of stressed adrenalectomized animals attain a size greater than that following adrenalectomy alone.[6] In order to assay the degrees of influence of various steroid hormones on lymphatic tissue weight it is necessary to administer graded amounts of these compounds to adrenalectomized animals. In this way, the stressor effects of hormone administration are eliminated, and the actual capacities of various hormones to produce graded degrees of involution or growth of lymphatic tissue may be assayed.

Although the actual procedure for performing these assays must be given in detail elsewhere, it may be well to mention, at this point, that the maximum increase in size of the thymus and lymph nodes following adrenalectomy in non-stressed mice occurs by the fourth day after operation.[7] Thereafter, there is no further increase in the size of lymphatic organs. Consequently, graded doses of the hormone to be assayed are given daily to adrenalectomized mice for a period of four days. Twelve hours after the last dose of hormone, the animals are killed; the lymphatic organs are weighed, and compared to the weights of the same organs of intact animals of the same age and sex and to those of four-day non-treated adrenalectomized animals.

The results of such assays demonstrate that lymphatic organ mass is delicately influenced by the amount of hormone available to an animal. Over a considerable dosage range, there is a highly significant inverse relationship between the concentration of certain adrenocortical hormones and the amount of lymphatic tissue.

Comparison of the potencies of adrenocortical steroids to moderate lymphatic tissue growth. The above method was utilized to obtain the potency ratios of various closely related adrenocortical steroids.[8] For example, hydrocortisone is 3.5, 4.6, and 6.8 times as potent as cortisone, and Kendall's Compounds B (corticosterone) and A (11-dehydrocorticosterone) respectively.[8] On the other hand, Reichstein's Substances L (*allo* Pregnane-3(B):17(B):diol-20-one) and S(4-Pregnene-17(B):21-diol-3:20-dione) do not induce any significant changes in weight of thymus. DCA (11-desoxycorticosterone acetate) produces true growth of thymus and lymph nodes and Substance L produces a questionable growth (not statistically significant) of lymph nodes but not of the thymus of treated adrenalectomized animals.

In summary, certain adrenocortical hormones exert a highly quantitative moderating influence on the growth of lymphatic tissue. The degree of this influence depends upon the chemical structures of these compounds and their concentrations in the tissues.

Mechanisms of action of hormonally mediated lymphatic tissue involution. There are two obvious mechanisms by which the amount of lymphatic tissue can be decreased. First, the rate of destruction of lymphocytes can become greater than the rate of production and second, the rate of production of these cells can be diminished. In the majority of studies emphasis has been placed upon the ability of adrenocortical steroids to increase the rate of lymphocyte destruction (lymphocytolysis). On the other hand, it was pointed out some years ago that adrenocorticotropin (ACTH) and adrenocortical hormones (adrenocortical extracts and the purified steroid hormones, cortisone and hydrocortisone) not only exert lymphocytolytic effects but also suppress lymphocytopoiesis.[9] The initial rapid loss of weight of lymphatic organs which follows hormone treatment was attributed to destruction of lymphocytes.[9] The period of time, however, after hormone treatment during which cellular destruction takes place is relatively brief, whereas mitosis is depressed for a long period following a single injection of an adrenocortical hormone.[9] Thus, the overall phenomenon of acute involution must be attributed to both of these mechanisms of action.[10]

Recent work in the author's laboratory has shown that these two mechanisms may act independently, *i.e.*, if animals are given daily injections of large doses of ACTH, cortisone or hydrocortisone for a period of days, almost no evidence of lymphocytolysis may be seen, although lymphatic tissue involution is maintained for the entire period of treatment.[10] This maintained lymphatic tissue atrophy (as contrasted to rapid involution and rapid reconstitution) is characterized histologically by a suppression of mitosis and by a monotony of small pycnotic cells.

Investigation of the cytological alterations in the various types of lymphocytes in the lymphatic organs of hormone-treated animals at different periods following the administration of hormone has revealed that lymphocytes exhibit differential morphological responses to adrenocortical hormones.[9, 11] The reticulo-endothelial cells and the immature lymphocytes (reticular lymphocytes, according to the classification of Sundberg and Downey,[12]) are most resistant to the lymphocytolytic effects of adrenocortical secretions.[9] This appears to be the case whether the hormones are given in single injections or for prolonged periods.[10] On the other hand, many of the more mature lymphocytes bud their cytoplasm, some become pycnotic and still others undergo karyorrhexis.

It is not as yet known whether these changes represent progressive stages of cellular injury, whether they are due to different concentrations of hormone acting on different cells or whether mature lymphocytes have differential susceptibilities to similar amounts of hormone. There is considerable evidence, however, that pycnosis does not always indicate cellular death and that pycnotic cells do not necessarily undergo karyorrhexis (unpublished data). Thus, if animals are subjected to prolonged treatment with large doses of adrenocorticosteroids for a period of six days, the atrophic lymphatic organs contain large numbers of pycnotic cells with no accompanying evidence of karyorrhexis.[2, 10] Following cessation of hormone treatment the pycnotic cells recover and begin to exhibit their former typical chromatin distribution before evidence of resumed homoplastic and heteroplastic proliferation is observed.[10]

In summary, acute involution of lymphatic organs is a result of several different adrenocorticosteroid actions at the cellular level, *i.e.*, budding of lymphocyte cytoplasm, pycnosis, karyorrhexis and inhibition of homoplastic and heteroplastic lymphocytopoiesis. There appears to be a differential susceptibility of the cells. Immature and "stress" lymphocytes (Type II lymphocytes of Downey) are more resistant to lymphocytolysis and mature small lymphocytes are more susceptible to pycnosis and destruction.

The basic mechanisms by which corticosteroids produce these lymphocyte alterations are not shown. In *in vitro* preparations, it has been shown that shedding of lymphocyte cytoplasm, shrinkage of the nuclei and karyorrhexis can be produced by cortisone and hydrocortisone.[11] Complete destruction (lymphocytolysis) of the lymphocyte, however, has not been adequately demonstrated in *in vitro* preparations. Apparently, lymphocytolysis has only been produced *in vitro* when some co-factor derived from lymphatic tissue is present.[13] Further investigation of the effects of various adrenocortical steroids on lymphocytes in isolated systems should increase our knowledge concerning the most fundamental mechanisms of action of these hormones at the cellular level.

Adrenocortical hormonal influence on antibody formation. Adrenocortical hormones might influence the release and/or synthesis of antibody protein by mechanisms other than those concerned with the effects of these agents on lymphatic tissue involution and growth. Since there is no information at present concerning such other mechanisms it would appear more fruitful, at this time, to analyze the interrelationships which may exist between two superficially dissimilar adrenocortical hormonal effects, *i.e.*, on the growth of lymphatic tissue and on antibody formation.

The reticulo-endothelial cells and their more differentiated offspring such as lymphocytes and plasma cells have been implicated as the principal cellular sites of antibody synthesis.[14, 1] The same group of cells are markedly affected by adrenocortical hormones, *vide supra.* The suggestion has been made that an anamnestic response may occur as a result of the shedding of antibody-containing lymphocyte cytoplasm.[15] Several observers, however, have failed to find that administration of ACTH or cortisone induces an anamnestic response[16, 17, 18] and, further, have questioned whether nonspecific stressors elicit such responses.[19] Other reports support the finding that preformed antibody is released by adrenocortical hormones.[20, 21, 22]

The question has been raised as to whether the lymphocyte or the plasma cell is the primary site of antibody synthesis. An answer to this problem is difficult and depends ultimately upon whether it can be demonstrated that either one cell or the other is solely responsible for antibody synthesis and, further, whether or not these cells belong to truly different cell lines or merely represent morphological alterations of a single-cell type. Certainly, the facts indicate that lymphocytes contain antibody protein [23, 24, 25, 26] and recent evidence conclusively demonstrates that they synthesize antibody *in vitro*.[26] Further, when one considers the fact that the number of lymphocytes in the body is vastly greater than the number of plasma cells of the Marschalko type, it is obvious that, even if lymphocytes synthesize less antibody per cell than the typical plasma cells, the vast majority of antibody protein supplied to the organism must still be derived from lymphocytes.

Although there is conflicting evidence concerning adrenocortical mediated release of antibody protein, it has been conclusively demonstrated that the amount of antibody in the blood is decreased in immunized animals treated with sufficient amounts of ACTH, cortisone or hydrocortisone.[27, 28, 29, 30, 10] So far, however, there is no evidence that antibody synthesis is inhibited in normal human experimental subjects or in patients treated with doses of ACTH or cortisone sufficient to produce alleviation of symptoms of asthma, arthritis, *etc.*[31, 32, 33]

It seemed to the reviewer that the antibody suppressing effects of adrenocortical hormones could, like numerous biological processes, be a dose-response phenomenon. By using a quantal response technique to ascertain the extent of antibody suppression, graded single doses of antigen and graded doses of ACTH or cortisone were administered to mice.[10] These related to the amount of antigen and hormone administered; *i.e.*, the greater the immunizing dose, the greater the amount of hormone required to suppress antibody synthesis. In spite of continued hormone treatment, however, some antibody eventually appears in the blood and, in order to suppress its formation completely, dosages of adrenocortical hormones far greater proportionately than those used therapeutically in human diseases are required.[10] With respect to the mechanism of action of adrenocortical hormones in allergic diseases, it is significant that, in spite of maximum inhibition of antibody synthesis as determined by measuring circulating antibody, sufficient antibody was formed so that anaphylactic shock was produced by a challenging dose of the homologous antigen.[10] This finding has led the author to agree with Mirick[32] that, "the beneficial effects of ACTH and cortisone in treating hypersensitive states and related diseases in man are not to be explained by suppressed antibody production."

At first glance, it would appear that the suggestion that adrenocortical secretions enhance the release of antibody conflicts with the finding that antibody titers may be decreased by these same agents. Halpern and his coworkers[22] considered this problem and performed the most definitive investigation on the effects of cortisone on antibody titer. These investigators immunized rabbits to ovalbumen and then administered a sufficient amount of antigen to the immune rabbits to reduce the circulating antibody to zero levels. When cortisone was administered to a group of animals treated in this manner, they responded with a rapid increase in circulating antibody, whereas the antibody titers of animals which did not receive the hormone remained at zero levels until 48 hours. Although cortisone increased the antibody titer immediately, it prevented the development of the greater amount found at later periods (4–7 days) in non-hormone treated animals. Halpern, *et al*, conclude that the rapid increase in antibody titer in the cortisone treated group was preformed antibody released from lymphocytes. Thus, they suggest that adrenocortical hormones exert two effects: They enhance antibody release, and also tend to diminish synthesis. The fact that cortisone inhibits the secondary response was confirmed by Fischel, *et al.*[30]

In view of the facts that the rate of disappearance of antibody passively administered to rabbits was not enhanced by cortisone treatment[34, 35] and that the secondary response can be inhibited by adequate doses of adrenocortical hormones,[30, 22] it is clearly established that inhibition of antibody synthesis rather than increased catabolism is the basic mechanism by which adrenocortical hormones suppress the amount of circulating antibody.

There is a very close correlation between inhibition of antibody formation and the maintained atrophy of lymphatic tissue. Stoerck and Eisen[36] called attention to the fact that pyridoxin deficiency in either intact or adrenalectomized mice was accompanied by a diminished rate of synthesis of antibodies and also by a depression of lymphocytopoiesis. Similarly, numerous observers have reported the coincident lymphatic tissue atrophy and inhibited antibody formation in irradiated animals.[37] In addition, adrenocortical hormonal suppression of antibody synthesis is accompanied by a decreased rate of lymphocytopoiesis.

The relation between lymphatic tissue involution and inhibition of antibody synthesis was investigated by giving dosages of ACTH or cortisone that were sufficient to suppress antibody formation following administration of one dose of antigen.[10] After giving antigen (0.05 cc horse serum) and hormone treatment daily, further hormone treatment was stopped at intervals of 9, 12 and 17 days. The rate of synthesis of antibody, the rate of reconstitution of lymphatic organ weights and the cytological changes in lymphatic organs were then studied at intervals of 24 hours after cessation of the hormone treatment. Antibody appeared in the circulation at 48 hours and by 72 to 96 hours was equivalent in amount to that found in similarly immunized non-hormone treated control animals. During the period of treatment, when antibody synthesis was suppressed, the lymph nodes contained apparently unaffected reticulo-endothelial cells, reticular lymphocytes and pycnotic lymphocytes. There was, however, little evidence of cellular debris and an almost complete suppression of mitosis. When hormone treatment was stopped, however, heteroplastic lymphopoiesis began before resumption of mitotic division became marked.[10]

The correlation between the resumed heteroplastic differentiation of lymphocytes and synthesis of antibody protein led the reviewer to suggest that antibody synthesis takes place during the differentiation of lymphocytes from fixed or free reticulo-endothelial cells.[1, 10] This "heteroplastic theory" suggests that antibody production is similar to other types of specialized protein synthesis in other sites such as that found in erythropoiesis, spermatogenesis, cutaneous epithelium, *etc.*

Influence of Adrenocortical Hormones on Inflammation

Early in the investigations of the effect of adrenocortical hormones on inflammation it was recognized that cortisone moderated the inflammatory response regardless of the nature of the inflaming stimulus.[38] For purposes of semantic convenience, this property has been designated the antiphlogistic action of adrenocortical hormones.[38] The degree to which inflammation is moderated by adrenocortical hormones is determined by two reciprocally interrelated factors.[10] First, the inflammatory response is directly proportional to the strength of the inflaming stimulus; and second, it is reciprocally proportional, at least over a considerable range of doses, to the amount of antiphlogistic hormone available at the site of inflammation.

Among several closely related steroid compounds, assayed in the author's laboratory by a method described elsewhere,[10] it was found that only Compounds B, E, F, and Substance S manifested antiphlogistic activity.[39] The potency ratios of these compounds were:

F/B	2,440
F/E	76.8
E/B	31.8

Since the linear regression line obtained for Compound S was not parallel to those obtained for the other effective compounds, no potency comparison could be made. The amount of Compound S required for antiphlogistic effectiveness, however, was far greater than that of any of the other effective compounds.

Structural activity relationships deduced from these data indicate that a hydroxy group at position C-17 and the alpha-beta unsaturation of ring A is essential for antiphlogistic effectiveness. When oxy or hydroxy groups at position C-11 are also present the antiphlogistic potency is greatest. Studies of other molecular configurations are now in progress.

Mechanism of Antiphlogistic Action of Adrenocortical Hormones. It has been suggested by Levine[40] that adrenocortical hormones and autonomic effector agents synergize each other in order to maintain optimal local blood supply. Levine has placed emphasis on this influence of cortisone as an explanation for its antiphlogistic effects. The decrease in amount of fibrin which was observed at the inflammatory site in cortisone-treated animals was attributed to the capacity of this compound to induce a decrease in the level of circulating fibrinogen.[41] Ungar called attention to the fact that cortisone inhibits the action of profibrinolysin and that this effect might in turn depress the inflammatory response. Several investigators found that adrenocortical extracts and also ACTH and cortisone inhibited the action of hyaluronidase.[42, 43, 44] Other investigators[45, 46] have suggested that the antiphlogistic effect of cortisone results from the capacity of this hormone to inhibit synthesis of ground substance. Although each of the hormonal effects mentioned above may influence certain aspects of the inflammatory response, it is likely that a still more fundamental function of the hormone may exist which, in turn, would influence each of these more superficial phenomena.

Acute inflammation includes a group of phenomena initiated by cellular injury which triggers a chain reaction composed of successive stimuli, each of which in turn initiates unique responses. Thus, adrenocortical hormones could prevent inflammation by inhibiting the inflaming effectiveness of the initiating stimulus or by interrupting the ensuing chain reaction. Attention should be directed, therefore, to possible influences of these hormones on the release, removal, and inflaming effectiveness of substances released from injured cells which initiate the inflammatory response.

Administration of cortisone does not prevent the release of anaphylotoxins in generalized anaphalactic shock or in local anaphylaxis[47] or *in vitro*.[48] Apparently cortisone does not inhibit release of histamine following administration of histamine liberating drug, but does diminish the expected histamine flare.[49]

Although adrenocortical hormones do not completely inhibit the release of phlogogenic substances, they may markedly diminish the amount of these agents elaborated at the site of inflammation. For example, Menkin,[50] has demonstrated that cortisone diminished the amount of leukotoxin released in experimentally induced inflammation.

Recent investigators in the author's laboratory (Dougherty and Schneebeli, unpublished data) indicate that the shedding of mast cell granules with a consequent release of heparin may bind histamine and inhibit the inflaming effectiveness of the latter agent. Although it is reported that adrenocortical hormones enhance the shedding of mast cell granules,[51] we have not found that this phenomenon occurs consistently nor is it entirely dependent upon the presence of the adrenal cortex. The combining capacity of heparin for certain amines which initiate inflammation may play a fundamental role in adrenocortical inhibition of inflammation. The general significance of this phenomenon, however, cannot as yet be evaluated.

Regardless of the basic mechanism by which adrenocortical hormones exert their effects on the phlogogenic substances, it seems clear that they inhibit inflammation primarily by suppressing progressive cellular damage and thereby interrupt the chain reaction which follows an inflammatory stimulus. There is experimental evidence that progressive fibroblastic damage in an area of inflammation is inhibited by administration of the antiphlogistic adrenocortical hormones.[47]

In summary, inflammation could be inhibited by several closely related mechanisms of adrenocortical hormone actions. Which particular influence, if any, of these is the most fundamental mechanism of antiphlogistic action has not been determined.

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THE EFFECT OF ADRENAL CORTICAL HORMONES ON EXPERIMENTAL INFECTION

By Harry J. Robinson and Amelia L. Smith
Merck Institute for Therapeutic Research, Rahway, N. J.

Studies concerning the effect of adrenal cortical hormones on infectious disease have attracted considerable attention during the course of the past two years. Published reports indicate that large doses of adrenocorticotropic hormone (ACTH), cortisone, or Compound F lower the resistance of animals to certain infectious agents. This appears to be well established for experimental pneumococcal[1-3] and streptococcal infections,[4-5] tuberculosis in the mouse, rat, and guinea pig,[6-11] rabbit syphilis,[12] brucellosis,[13] *Coccidioides immitis* infections,[14] and a number of virus infections in animals.[15-19] Relatively few reports have appeared concerning the influence of these agents on human infections[20-24] and, hence, much remains to be learned regarding the action of adrenal cortical hormones on infectious disease in humans.

The present communication is primarily concerned with the effect of ACTH and cortisone on certain defense mechanisms known to play a role in resistance to infection. As a working model, a pneumococcal infection in rabbits was employed. Under these conditions, it was possible to study the effects of the hormones on a number of the defense mechanisms concerned with resistance to infection as well as the survival time and rate.

Materials and Methods

Details of the materials and methods employed in this study have been presented elsewhere and, hence, only a few essential facts will be given here.[25] Infection was produced by injecting 0.1 cc. of a diluted pneumococcal culture intradermally into the shaved abdomen of each rabbit. The culture was grown at 37°C in blood broth for six hours, and then diluted with broth so that the inoculum for each rabbit contained approximately 250,000 to 300,000 pneumococci. The size of the skin lesion resulting from the pneumococcal infection was measured by tracing the periphery of the lesion on transparent paper. Methods for blood-culture studies, protective antibody titration tests, and the preparation of tissues for microscopic study are given elsewhere.[25]

Cortisone* was administered subcutaneously once daily in doses of 10 mg. per rabbit per day. Therapy was started seven days before the pneumococcal infection and then continued until death of the rabbit or for an additional 10 days after the bacterial injection. ACTH† was injected subcutaneously, every hour, night and day, in doses ranging from 0.25 mg. to 1.0 mg. per injection. Therapy was started two days before infection and continued until death of the animal. With these doses of cortisone and ACTH, marked metabolic and tissue changes characteristically produced by these agents were noted in all rabbits. Hence, it is apparent in this study that large doses of the hormones were employed. Since the effects of cortisone and Compound F were essentially the same, the results with cortisone will be presented primarily.

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* Cortone, Merck.
† Merck, equivalent to Armour Standard La-1-A.

Results

Before discussing the results of the studies concerning the mechanism of action of cortisone in this infection, it seems desirable first to outline briefly some of the effects of these agents as noted by gross observation.

Survival Time. Essentially all of the control animals survived the infection over a 10-day observation period. In contrast, most of the rabbits receiving cortisone, Compound F, or ACTH died within a similar observation period (TABLE 1).

TABLE 1

EFFECT OF CORTISONE AND ACTH ON SURVIVAL OF RABBITS INFECTED WITH *Diplococcus pneumoniae*

Days after infection	Per cent survival		
	Controls	Cortisone*	ACTH†
1	100	100	97
2	100	92	50
3	100	60	30
4	100	30	20
5	100	20	0
6	98	20	0
7	98	20	0
8	98	20	0
9	98	20	0
10	98	20	0

* 10 mg. cortisone injected once daily for 7 days before infection and continued for 10 additional days.
† 1.0 mg. ACTH injected subcutaneously every hour for 2 days before infection. Treatment continued for 5 additional days.

TABLE 2

EFFECT OF CORTISONE, COMPOUND F AND ACTH ON THE SIZE OF THE SKIN LESION

Days after infection	Size of skin lesion area in sq.cm.			
	Controls	Cortisone*	Compound F†	ACTH‡
1	30	80	95	10
2	48	120	130	10
3	50	130	130	0
4	52	130	130	—
5	50	130	130	—
6	42	—	—	—

* Cortisone injected subcutaneously, 10 mg. per rabbit per day. Treatment started 7 days before infection and continued for 5 additional days.
† Compound F injected subcutaneously, 10 mg. per rabbit per day. Treatment started 7 days before infection and continued for 5 additional days.
‡ ACTH, 1 mg. every hour, subcutaneously. Treatment started 48 hours before infection and continued for 4 days.

Gross Appearance of Skin Lesion. There was a marked difference in the gross appearance of the skin lesions in the control and treated animals. The lesions in the untreated rabbits developed slowly and at the 48 hours observation period were characterized by the formation of a localized pouch approximately 50 sq.cm. in diameter (TABLE 2). In the cortisone- and Compound F-treated animals, the lesions spread rapidly and, at the 48-hour period, the entire abdomen was frequently involved and the inflamed area had the appearance of a thickened erythematous placque. In contrast to both the controls and the cortisone-treated animals, there was little or no evidence of gross inflammation at the bacterial injection site in rabbits receiving ACTH. Thus, in the doses employed, ACTH essentially suppressed the inflammatory reaction completely, as judged by gross observation.

Factors Concerned with Altered Resistance to Infection

Effect of Cortisone on Pneumococci. When pneumococci were grown in the presence of cortisone or Compound F, there was no apparent alteration in the rate of growth, virulence, or morphology of the bacterial cells. Moreover, pneumococci grown in blood obtained from rabbits undergoing intensive ACTH or cortisone treatment appeared normal in all respects. Hence, the effects of these hormones appear to be solely upon the host.

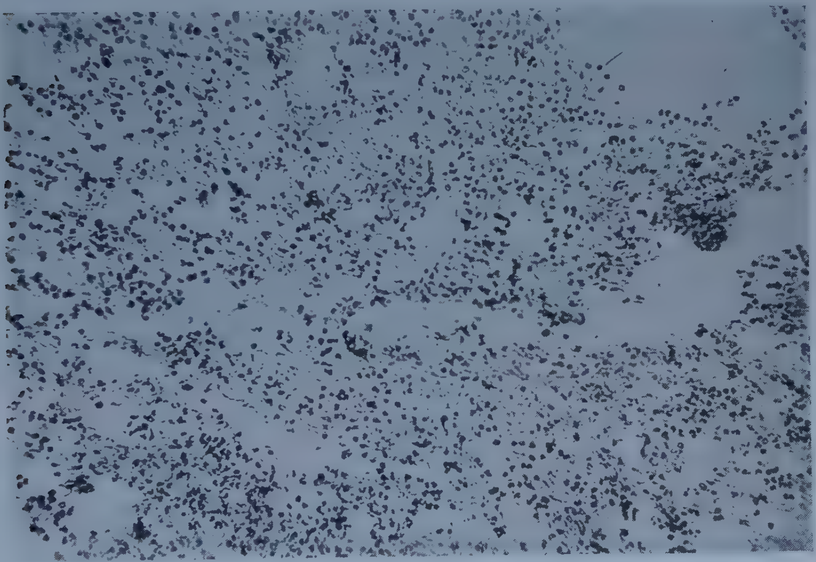

FIGURE 1. Photomicrograph of inflammatory reaction in control rabbit 12 hours after bacterial injection. × 440. Reproduced from "The Suprarenal Cortex" published in U.S.A. by Academic Press, Inc., through the courtesy of the Colston Research Society and University of Bristol, England.

Microscopic Findings at Injection Site. In the control animals, polymorphonuclear leucocytes were found at the injection site within 5 to 10 minutes following the pneumococcal injection. The number of cells increased rapidly and, within 8 to 10 hours, numerous leucocytes infiltrated the area. Many pneumococci were noted within the leucocytes, and relatively few bacterial cells were observed in the tissue spaces. Within 48 hours after the bacterial injection, the exudate was massive and consisted primarily of disintegrated white cells, edema fluid, and tissue debris.

The most outstanding effect noted in the animals treated with ACTH or cortisone was the delay in the mobilization of neutrophilic leucocytes (FIGURES 1 and 2). At the 2–4-hour observation period, there were relatively few leucocytes at the injection site in either the ACTH- or cortisone-treated animals as compared to the control animals. Equally striking was the relatively large number of unphagocytized pneumococci in the tissue spaces. Subsequently however, in the cortisone-treated animals, large numbers of polymorphonuclear leucocytes infiltrated the injection site and, at the 48-hour observation period, the microscopic findings were similar to that noted above for the controls. In the ACTH-treated rabbits, the inflammatory response was suppressed over a longer period of time and, frequently, the animals died as a result of overwhelming sepsis with little evidence of reaction at the injection site. It is apparent

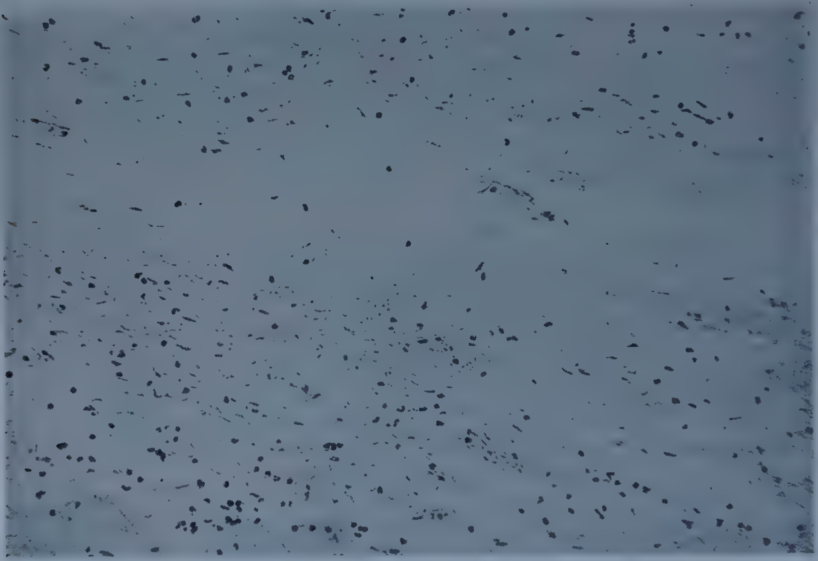

FIGURE 2. Photomicrograph of inflammatory reaction in cortisone-treated rabbit 12 hours after bacterial infection. × 440. Reproduced from "The Suprarenal Cortex" published in U.S.A. by Academic Press, Inc., through the courtesy of the Colston Research Society and University of Bristol, England.

TABLE 3

BLOOD CULTURE STUDIES IN CORTISONE, COMPOUND F, AND ACTH TREATED RABBITS INFECTED WITH *Diplococcus pneumoniae* TYPE 1

Hours after injection	Number pneumococci per cc. of blood			
	Controls*	Cortisone†	Compound F†	ACTH‡
0	0	0	0	0
2	0	0	0	0
4	0	200	400	300
12	100	600	700	1,200
24	300	9,000	12,000	14,000
48	1,800	∞	∞	∞
72	2,800	∞	∞	∞
96	400	∞	∞	∞
120	0			
144	0			

* Controls, no treatment.

† Cortisone and Compound F, 10 mg./rabbit/day. Treatment started 5 days before infection and continued for 3 additional days.

‡ ACTH, 1 mg. every hour. Treatment started 48 hours before infection and continued for 3 additional days.

therefore that one of the important defense mechanisms altered by these hormones is an interference with the rate of mobilization of polymorphonuclear leucocytes at the injection site.

Blood-Culture Studies. As expected from the foregoing microscopic findings, the control rabbits developed a transient bacteremia which had its onset about four hours after the bacterial injection. The bacteremia reached a peak of 3000 cells per cc. of blood at the 72-hour observation period and then gradually subsided (TABLE 3). The hormone-treated animals developed a bacteremia somewhat earlier than the control rabbits, although this was not a consistent finding. More striking was the rapid increase in the number of pneumococci in the blood. In most rabbits, an overwhelming bacteremia developed within a 4–5-day period. It seems most likely that the uninhibited multiplication of pneumococci at the injection site will account, in part, for the overwhelming sepsis found in the hormone-treated animals.

TABLE 4

DISAPPEARANCE OF PNEUMOCOCCI FROM BLOOD OF RABBITS FOLLOWING INTRAVENOUS INOCULATION

Hours after inoculation	Number pneumococci per cc. blood	
	Controls*	Cortisone†
0	10×10^6	12×10^6
1	1.2×10^6	1.4×10^6
2	3×10^5	7×10^5
3	21×10^4	3×10^4
4	12×10^4	8×10^4
5	1.6×10^3	9×10^4
6	1.2×10^3	8.1×10^4
7	8×10^2	8.6×10^4
24	6×10^3	1.2×10^5
48	2×10^3	∞
72	0	∞

* Controls, no treatment.
† Cortisone, 10 mg. per rabbit per day for 5 days before infection. Treatment continued once daily for 3 days after infection.

TABLE 5

PROTECTIVE ANTIBODY TITER IN SERUM OF RABBITS INFECTED WITH *D. pneumoniae* TYPE 1

Days after infection	Serum dilution required to protect 100% of infected mice*	
	Control rabbits	Cortisone rabbits†
1	x	x
2	x	x
3	x	x
4	1:64	x
5	1:256	1:8
6	1:512	1:16

* x = No protection with undiluted serum.
Serum mixed with 10 L.D. of pneumococci for 30 min. before intraperitoneal injection in mice.
† Cortisone, 10 mg. per rabbit per day. Treatment initiated 5 days before infection.

Reticuloendothelial System. The removal and destruction of virulent bacterial cells by the fixed macrophages of the reticuloendothelial system represents a major feature of the defense mechanism in infection. In view of the results of the blood-culture studies, it was of importance to determine the effect of cortisone and ACTH on this sytem. When pneumococci were injected intravenously into normal rabbits, the bacterial cells were rapidly cleared from the blood and the animals survived the infection (TABLE 4). When ACTH- or cortisone-treated animals were given a similar intravenous injection, the rate of disappearance of the pneumococci from the blood was similar to that of normal rabbits during the first few hours but, thereafter, the number of bacterial cells rapidly increased and overwhelming sepsis occurred. These findings suggest, among other things, that the ability of the fixed macrophages to destroy virulent pneumococci is altered in some manner. Hence, a second important defense mechanism appears to be modified by large doses of cortisone or ACTH.

TABLE 6

EFFECT OF PENICILLIN IN INFECTED RABBITS RECEIVING CORTISONE

Days after infection	Per cent survival of infected rabbits		
	Controls	Cortisone*	Cortisone + Penicillin†
1	100	100	100
2	100	80	100
3	100	60	100
4	98	30	100
5	97	20	100

* Cortisone, 10 mg. per rabbit per day.

† Penicillin, 3000 units per rabbit per day injected at time of bacterial injection and continued once daily for 5 days.

Formation of Protective Antibodies. It has been reported previously that cortisone depresses antibody formation.[26] The findings in the present study also show that cortisone delays the appearance of circulating protective antibodies in the sera of rabbits infected intradermally with a Type I pneumococcus (TABLE 5). In view of the importance of antibodies to the phagocytic function of the reticuloendothelial system, the effect of cortisone and ACTH on antibody formation appears to be particularly significant.

It was of interest to note that, if cortisone-treated rabbits were passively immunized with small amounts of antipneumococcal serum, the animals were able to combat the infection in a normal manner. This was found to be the case even 24 hours after infection was initiated. The macrophages of the reticuloendothelial system rapidly removed and destroyed the pneumococci. Moreover, if penicillin was injected prophylactically in doses of 3000 units per rabbit per day, all cortisone- and ACTH-treated animals survived (TABLE 6). Hence, it is possible to control this infection in animals receiving large doses of the hormone despite the impaired defense against infection.

Discussion

The present brief review showed that large doses of ACTH, cortisone, or Compound F lowered the resistance of white male rabbits to a pneumococcal infection. It was found that cortisone had no effect on the pneumococcus *per se*, and hence the deleterious effect of these hormones on the resistance of rabbits to infection appeared to be associated with altered host resistance. Three factors of importance to the resistance of animals to infection appear to be altered by large doses of ACTH and cortisone. First, rabbits receiving cortisone or ACTH are unable to mobilize polymorphonuclear leucocytes at a normal rate and hence permit the pneumococci to multiply rapidly and invade the blood stream early. Second, once the organisms enter the circulating blood, the fixed macrophages of the reticuloendothelial system fail to remove and destroy the bacterial cells at a normal rate. Third, the formation of antibodies are depressed by these agents; hence deprive the animal of an important defense against the invading bacteria. It should be borne in mind that all of the findings reported in the present review were obtained with large doses of ACTH and cortisone. There is experimental evidence that cortisone, in the proper doses, will enhance the survival of infected adrenalectomized animals.[27] This beneficial effect does not appear to be related solely to the effect of cortisone on bacterial toxins, but actually represents a greater ability to combat infection.

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OBSERVATIONS ON THE EFFECT OF CORTICOSTEROIDS AND GROWTH HORMONE ON RESISTANCE TO EXPERIMENTAL PNEUMOCOCCAL AND INFLUENZA VIRUS INFECTIONS*

By Edward H. Kass, Marguerite M. Lundgren, and Maxwell Finland
Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard); Boston City Hospital; and Department of Medicine, Harvard Medical School, Boston, Mass.

Following the initial observation that the administration of ACTH to patients with pneumonia was followed by rapid defervescence and relief of symptoms without any demonstrable effect on the infecting agent,[1] studies were undertaken on the effects of ACTH and cortisone in experimental infections. These studies revealed differences between the effects of ACTH and cortisone on infections in mice.[2] Further investigations were then undertaken on some other pituitary and adrenal hormones as they became available to us. The results of these studies will be summarized.

Two experimental systems were employed. In the first, adult mice were given sufficient specific antiserum to protect half of them against about 50,000 LD_{50} of type 2 pneumococcus. Groups of such protected mice were infected with varying numbers of pneumococci, after treatment with one or another hormone. The survival time of mice receiving hormonal treatment was compared with that of control animals. Twelve days was arbitrarily chosen as the maximum survival time.

Even under these relatively weighted conditions, cortisone acetate (FIGURE 1), in various dosage schedules, shortened the mean survival time of the protected mice significantly. ACTH (Armour) did not significantly affect the mean survival time of the mice even in doses of 10 mg. every 12 hours. A highly potent form of corticotropin, obtained from Drs. Astwood and Raben and with 50 times the activity of ACTH (Armour), as measured by the effect on murine leucocytes, was also tested and it, too, did not significantly affect survival in these mice. Hydrocortisone acetate depressed resistance variably but the free alcohol of compound F was quite effective in depressing resistance in the passively immunized mice. Presumably, the difference between the two forms of 17-OH corticosterone is referable to slow hydrolysis of the acetate.

A small supply of corticosterone, obtained from Dr. Abraham White, also depressed resistance to infection significantly in this system. A highly purified growth hormone, prepared and provided by Drs. E. B. Astwood and M. S. Raben, likewise depressed resistance and, even in large doses, failed to reverse the effect of cortisone in depressing resistance to infection.

Not only was the mean survival time of the animals decreased by the steroids tested and by growth hormone, but, from FIGURE 2 it is seen that the LD_{50} dilution titer was increased, so that the mice succumbed to smaller numbers of bacteria than did the controls. Again, ACTH had no significant effect and the effect of cortisone was not reversed by growth hormone.

Influenza A infection in mice develops more slowly than pneumococcal infection. In the bacterial infection, most of the deaths occur within the first

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* Aided by a grant from the United States Public Health Service.

48 hours after inoculation of the organisms, whereas, in the viral infection, deaths seldom occur before the fifth or sixth day after intranasal instillation of the virus. If, for the purposes of calculation, survival is arbitrarily assigned

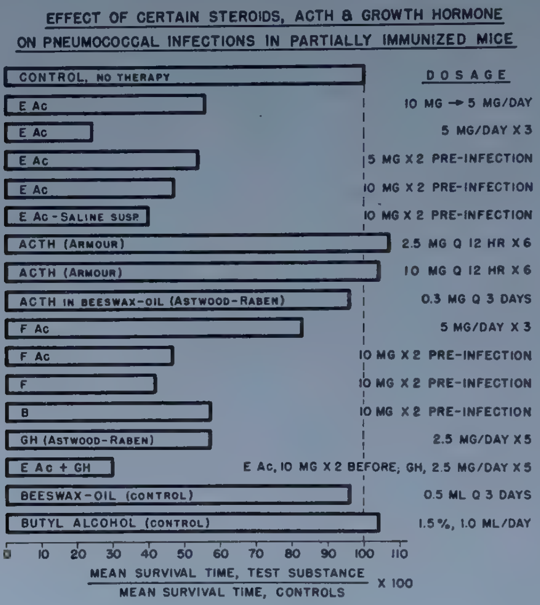

FIGURE 1

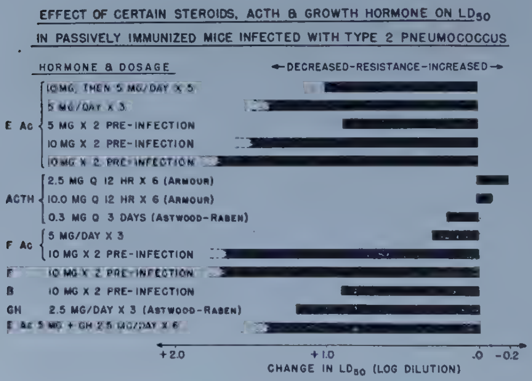

FIGURE 2

a value of 12 days, only a fairly marked effect would appear as a significant deviation from the control figures. Nevertheless, as FIGURE 3 shows, cortisone decreased the mean survival time, whereas ACTH (Armour) did not. Astwood and Raben's corticotropin in oil and beeswax reduced survival somewhat, although, as will be shown later, this preparation had no significant effect on the LD_{50}. Compound F and its acetate also slightly decreased the survival time of the mice infected with influenza virus, whereas growth hormone from two different sources and in varying dosages decreased the survival time only inconstantly. Here again, the effect of cortisone on survival time was not overcome by the administration of large doses of growth hormone.

The LD_{50} dilution of the viral suspensions (FIGURE 4) was increased as a result of administration of cortisone, so that otherwise inocuous concentrations of virus were lethal after pretreatment of mice with the hormone. On the other hand, ACTH in large doses, even in highly purified form, did not significantly alter the LD_{50}, whereas compound F depressed resistance to this virus. The effect of growth hormone was variable, but no significant difference from the controls was produced, and no evidence could be obtained that growth hormone overcame the effect of cortisone.

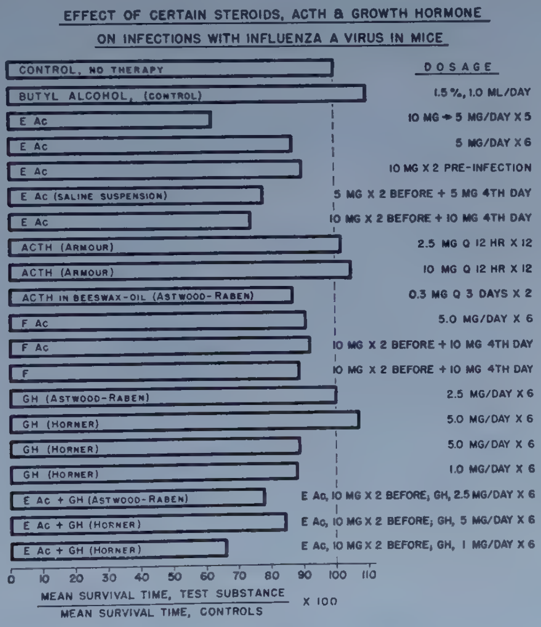

FIGURE 3

Quantitation of the influenza virus persisting in the murine lung after infection showed that the titers reached a maximum by the second to fourth days and diminished rapidly by the sixth day. Cortisone and hydrocortisone did not greatly increase the titers of virus in these lungs, but the effect was inconstant. Experiments to date indicate that injection of these steroids delays the disappearance of virus from the infected lungs.

It would appear from these experiments that cortisone and hydrocortisone decreased the capacity of the host to survive a given concentration of virus in the lungs, and that these hormones interfere with some mechanism by which viral particles are disposed of by the host.

Such an interference may also have been operative in some experiments carried out in rats which are ordinarily immune to influenza A virus. When large doses of this virus were instilled intranasally into rats, some multiplication of virus occurred but, within four to six days, the virus virtually disappeared from the lungs. Cortisone permitted viral concentrations to reach higher titers, and to persist longer than in control animals, although fatalities did not occur.

What are the possible implications of these experiments? There is evidence, from several sources, that the adrenal cortices of man generally produce 17-OH corticosterone and corticosterone, and it appears that greater amounts of the former than the latter may be produced.[3] The same seems to be true of oxen, dogs, cats, and guinea pigs.[4, 5] Rats and rabbits, on the other hand, produce corticosterone as the principal, if not the only, adrenocortical hormone.[5] There are, as yet, no data concerning the mouse. Also, it is not clear whether the type of adrenocortical hormone produced in any species may change following marked stimulation with exogenous ACTH.

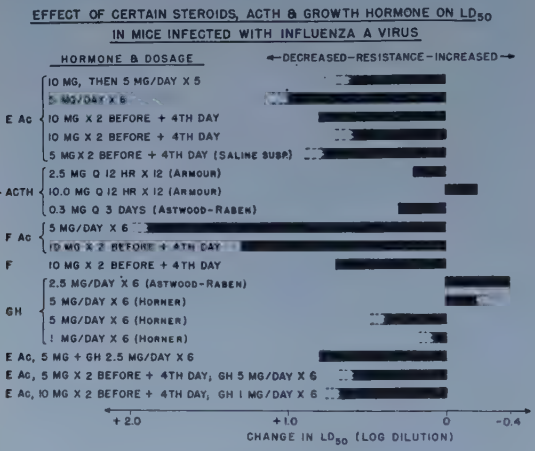

FIGURE 4

Although there are few comparative data concerning the responses of different laboratory animals to the effects of ACTH and cortisone, there is evidence that, in man, monkeys, rabbits, rats, and guinea pigs, large doses of ACTH act like cortisone in depressing resistance to infection or in altering one or another manifestation of responsiveness to inflammatory irritants or immunologically active substances. In mice and hamsters, on the other hand, ACTH in large doses does not depress resistance, whereas cortisone may do so. In man, corticosterone is not antiarthritic in concentrations comparable to effective doses of cortisone.

It is assumed that the antiarthritic effect of cortisone is related to the depression of resistance to infection through its anti-inflammatory action. Inasmuch as ACTH depresses resistance to infection in rabbits and rats, which are corticosterone secretors, either (1) the response of these animals to corticosterone is different from that of man; or (2) ACTH may, under extreme conditions of stimulation, cause the adrenal cortices of rabbits and rats to produce steroids other than corticosterone; or (3) corticosterone has an anti-inflammatory effect, which may be quantitatively less intense than that observed after the administration of the same amount of cortisone or hydrocortisone.

The present results suggest that corticosterone may be anti-inflammatory in its effect in mice and, also, that mice may produce an adrenal steroid that is different from compounds B, E, or F, assuming that the failure of ACTH to depress resistance is to be explained on these grounds alone. Further experimentation is obviously indicated to elucidate these points.

The failure of growth hormone to reverse the effect of cortisone was disturbing. Conceivably this, too, represents a species difference. The beneficial effects of growth hormone noted by Selye[6] were observed by him in rats that had developed spontaneous pulmonary infections during treatment with cortisone. In the present studies, purified growth hormone was obtained from two different laboratories, one of which also supplied some of the hormone used by Selye. The doses of growth hormone used here were large, but beneficial effects were not discernible. Nevertheless, preliminary experiments indicated that growth hormone might overcome to some degree the effect of cortisone on the weights of mice.

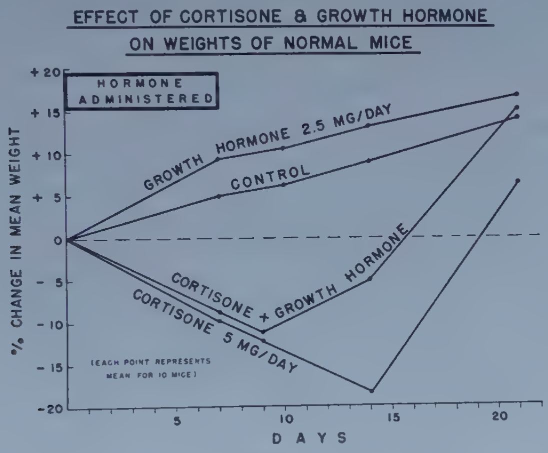

FIGURE 5

FIGURE 5 shows that mice receiving growth hormone grew slightly more rapidly than control animals, and that those receiving cortisone lost an appreciable amount of weight. As the effects of cortisone were wearing off, growth hormone seemed to induce more rapid recovery of weight than occurred in mice receiving cortisone without growth hormone, which suggests that growth hormone may overcome some of the effects of relatively small amounts of cortisone. This point will be tested further by attempting to obtain long-acting preparations of growth hormone, which might be more effective per unit weight, and by attempting to define conditions whereby a reproducible effect of cortisone on resistance to infection might be demonstrable even with smaller doses of cortisone.

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Discussion of the Paper

Doctor Erwin P. Vollmer (*Naval Medical Research Institute*): I should like to ask Dr. Kass how he would characterize physiologically the doses of cortisone he used on infected mice. It is my impression that these doses are in a range that would be lethal within a week or so. If this is so, it appears to me that the interpretations that have been made with regard to the harmful effects of cortisone in infection may have been overdrawn and require emphasis on the phrase "under the conditions of the experiment."

I should also like to add that, in mice infected with Japanese encephalitis-B virus, Doctor Hurlbut and I found augmented mortality rates only at dosages of cortisone that affected the weight curves of control mice; and that, in mice infected with pneumococci, aqueous adrenocortical extract exerted, if anything, a slight beneficial effect.

Doctor Kass: Dr. Vollmer's question is difficult to answer conclusively. Of course, all of the deductions are true "under the conditions of these experiments." It is worth pointing out, however, that two doses of 5 mg. of cortisone, given four hours apart, just before the introduction of pneumococci, depressed resistance to infection significantly. The effect was noticeable within the first 24 hours of the experiment. This amount of cortisone does cause losses of weight in the mice, but the weight loss during the first 24 hours is less than 2 per cent of the total body weight. Furthermore, no deaths are encountered in uninfected mice receiving this dose of hormone. As was pointed out in an earlier study,[2] ACTH failed to depress resistance to pneumococcal and influenza viral infections in doses that induced a greater loss of weight in the mice than did doses of cortisone that did depress resistance. We would readily agree with the implication that doses of cortisone that depress resistance must be sufficient to induce some physiologic evidences of hypercorticoidism. The same appears to be true in inflammatory diseases in man.

It should be pointed out that, in certain viral and protozoal infections, inanition confers some increased resistance on the host animal. Yet, in similar infections, cortisone induced decreased resistance, despite the loss of weight. Of further interest would be a study of the lethal effects of cortisone in large doses. We have the distinct impression from our observations, and from those of other workers, that, in many instances, the large doses of cortisone permit spontaneous, severe infections to arise in the host animals, and that these may contribute toward the lethal effect of cortisone.

Some aid for the tentative conclusions is also derived from indications that locally instilled cortisone or hydrocortisone, in amounts that produce no apparent hypercorticoidism throughout the host, will nevertheless decrease the local inflammatory response in the areas in which the hormone is relatively abundant and, presumably, thereby increase susceptibility of that area to infection.

It would thus appear that cortisone in reasonably large doses depresses resistance to infection; while in the mouse ACTH in large doses, which have similar metabolic effects, does not depress resistance. We would agree that these large doses of either agent would, by some definitions, be called "toxic" under such circumstances, but the effect of the hormone in these high doses is nevertheless relatively specific for mechanisms of defense in the host.

THE EFFECTS OF CORTICOSTEROIDS UPON TUBERCULOSIS AND PSEUDOTUBERCULOSIS*

By Charles LeMaistre, Ralph Tompsett, and Walsh McDermott
Department of Medicine, New York Hospital-Cornell Medical Center, New York, N. Y.

The effects induced by corticotropin and cortisone upon the course of tuberculous infections have been reported in a number of investigations involving several animal species including man.[1, 2] The interpretation of the nature of these effects has varied markedly. The study we have conducted in the last two years is a series of investigations related to the effects of these agents in humans, mice, guinea pigs, and rats. This study will serve as the basis for the following remarks.

Results of the study of short-term administration of cortisone and corticotropin to humans have been published and will be referred to only briefly.[3] Administration of either hormone to patients with tuberculosis resulted in amelioration of the signs and symptoms of acute illness. Defervescence promptly occurred in the febrile patients. In the laryngeal lesions, there was regression of edema, inflammation, and granulation tissue. In approximately half of the patients, the tuberculin test became negative. In all patients, there was return of the previous signs and symptoms immediately on withdrawal of the hormone, although return of cutaneous hypersensitivity was sometimes delayed as long as four weeks. No other significant changes were noted during the short period of hormone administration and long-term administration has not been attempted.

Hart and Rees have reported the enhancement of experimental murine tuberculosis as a consequence of cortisone administration.[4] This effect was most marked in the chronic infection but was also apparent in the acute infection. Our limited studies in mice have been primarily concerned with the effect of corticotropin upon the early development of the tuberculous lesion. The animals, sacrificed at varying periods from one hour to 21 days after infection, revealed findings which were indistinguishable from those of the infected controls. It can be stated that, in this experiment, corticotropin did not exert demonstrable effect upon the early development of experimental murine tuberculosis.[5]

The effects of excessively large doses of corticotropin and cortisone upon the initiation and early progression of severe experimental tuberculosis in the albino guinea pig exhibited slight, though definite, differences in the effects of these agents.[6] The character and extent of tuberculous involvement was examined before and during infections of 14 and 28 days duration. In the infection of 14 days duration, nodules at the site of injection appeared in all of the control and corticotropin-treated animals. As first described by Spain,[7] the formation of a nodule was less evident in the cortisone-treated animals, occurring in only one-half of these animals. In the infection of 28 days dura-

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* This study was supported (in part) by grants from The Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service; the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York; Charles Pfizer and Company, Brooklyn, New York.

tion, the character and extent of the tuberculous process was more uniform in the treated groups, and significant differences from the control animals were not evident.

In the rat, an animal natively resistant to progressive tuberculous disease, infection with either human or avian tubercle bacilli results in essentially identical, stable, tuberculous lesions. In the Sprague Dawley strain, Michael and his associates have reported a fulminating, rapidly fatal tuberculosis when animals experimentally infected with the human type bacillus were treated with cortisone.[8] We have confirmed this work in the Sprague Dawley rat with the human-type infection. In another albino rat, the Wistar strain, experimentally infected with the avian type bacillus, significant effects upon the character or extent of tuberculous lesions, or in the number of bacilli present in the lesions, as a consequence of the administration of either cortisone or corticotropin have not been observed. Therefore, in contrast with the results in the cortisone-treated Sprague Dawley rat infected with human type tubercle bacilli, we have been unable to demonstrate enhancement of an experimental avian tuberculosis by either cortisone or corticotropin in another strain of albino rat.

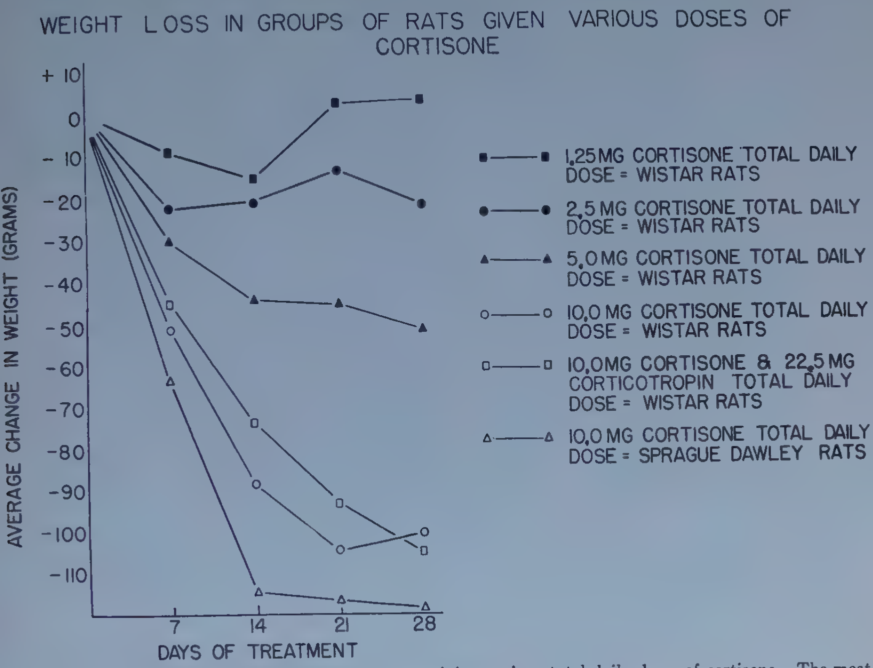
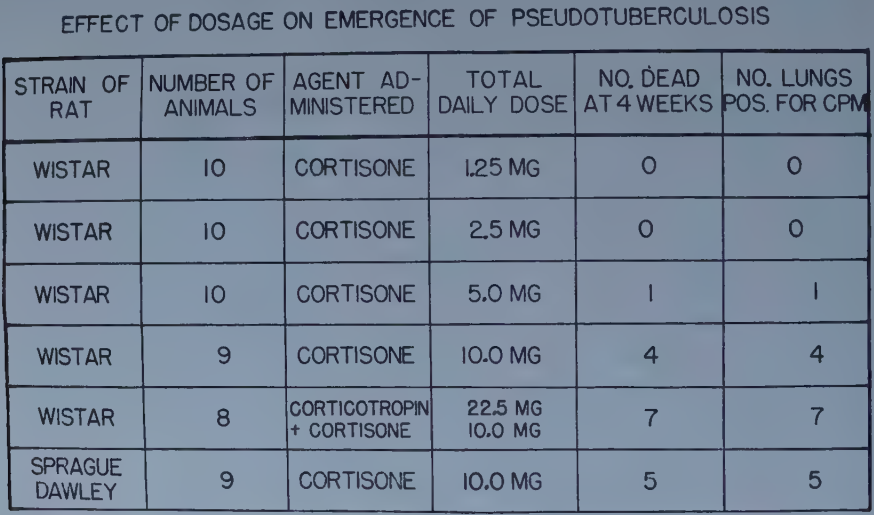


FIGURE 1. Average body weight change in rats receiving various total daily doses of cortisone. The most marked weight loss occurred in the animals that received the larger total daily doses of cortisone.

During the course of the investigation of the effects of large doses of these hormonal agents upon experimental tuberculosis in the rat, a fulminating, rapidly fatal infection was observed to occur with marked regularity in those animals receiving excessively large amounts of cortisone.[9] The etiologic agent of this infection was a microbial species not demonstrably a part of the usual bacterial flora of the rat and of unusually low virulence when artificially introduced into this host.

All animals that received saline or corticotropin showed no marked change in body weight. In cortisone-treated animals, however, weight loss occurred, frequently amounting to one-third the total body weight at the beginning of the experiment.

The amount of weight lost was related to the total daily dosage of cortisone. The animals which received the largest daily dose of cortisone had the most-marked weight loss, irrespective of whether corticotropin was administered concurrently (FIGURE 1).



EFFECT OF DOSAGE ON EMERGENCE OF PSEUDOTUBERCULOSIS

STRAIN OF RAT	NUMBER OF ANIMALS	AGENT ADMINISTERED	TOTAL DAILY DOSE	NO. DEAD AT 4 WEEKS	NO. LUNGS POS. FOR CPM
WISTAR	10	CORTISONE	1.25 MG	0	0
WISTAR	10	CORTISONE	2.5 MG	0	0
WISTAR	10	CORTISONE	5.0 MG	1	1
WISTAR	9	CORTISONE	10.0 MG	4	4
WISTAR	8	CORTICOTROPIN + CORTISONE	22.5 MG 10.0 MG	7	7
SPRAGUE DAWLEY	9	CORTISONE	10.0 MG	5	5

FIGURE 2. Frequency of pseudotuberculosis due to *Corynebacterium pseudotuberculosis murium* in rats given various total daily doses of cortisone. The disease emerged most frequently in the animals which received the largest total daily does of cortisone.

The dosage of cortisone was related also to the frequency of pseudotuberculosis (FIGURE 2). The disease appeared only in the animals receiving the larger doses of cortisone. Therefore, a positive correlation existed between the dosage of cortisone, the weight loss, and the development of pseudotuberculosis.

A representative example of the gross appearance of the pulmonary lesions observed in 41 of the 50 animals that received cortisone may be seen in FIGURE 3. These lesions were distributed throughout all lobes and consisted of pale-yellow coalescent areas of semisolid necrosis. The lesions consisted of an area of central necrosis surrounded by enormous numbers of structures with the appearance of microorganisms, which were present both grouped in colonies or as individual cells (FIGURES 4 and 5). A narrow and relatively ill-defined area of cellular infiltration, predominately by mononuclear cells, is present at the border of the lesion. This cellular reaction did not give the appearance of delimiting the progress of necrotizing lesions.

It is of considerable interest that the infection emerging in the cortisone-treated rats was consistently caused by the same microbial species, *Corynebacterium pseudotuberculosis murium.* A strain of microorganism with the bacteriologic and morphologic characteristics of *Corynebacterium pseudotuberculosis murium* was recovered only from the tissues of the cortisone-treated animals. The isolation of this microorganism was successful in 34 of the 39 instances in which it was attempted. Cultivation of this microorganism was attempted from the lungs, liver, spleen, and kidneys of 10 saline-treated rats, 30 corticotropin-treated rats, and 10 rats maintained in the animal room for three months before sacrifice. In all instances, the attempts were unsuccessful.

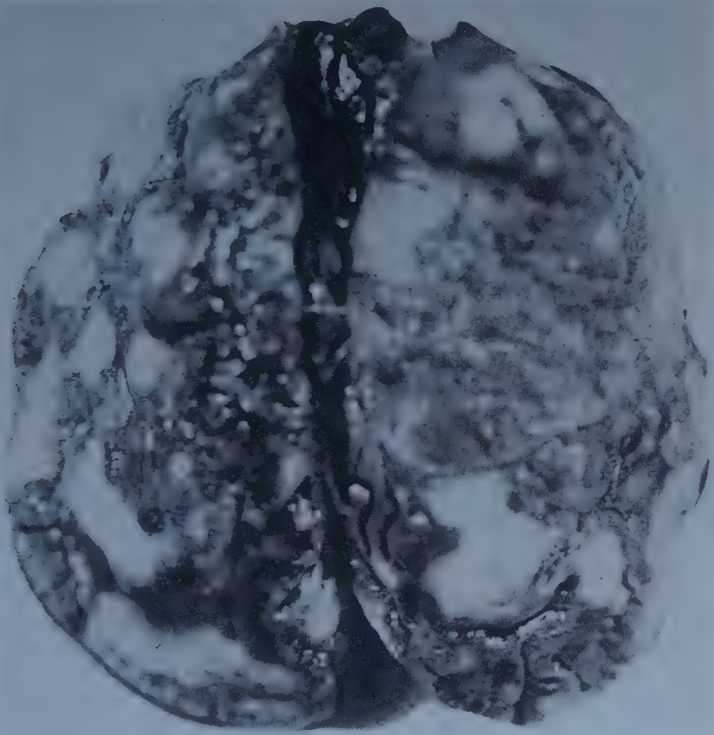


FIGURE 3. Lesions of the lung of a rat which had received excessive cortisone.

Therefore, none of the corticotropin-treated rats developed evidence of pseudotuberculosis, whereas infection due to the same strain of corynebacteria was demonstrated in over 80 per cent of the rats receiving excessive cortisone. Moreover, a special attempt was made to exclude the possibility of dosage effect making any similarity in the action of corticotropin and cortisone by the inclusion of a group of animals receiving 22.5 mg. of corticotropin daily in divided doses at eight-hour intervals. Even on such excessively large doses of corticotropin, there was no evidence of the development of pseudotuber-

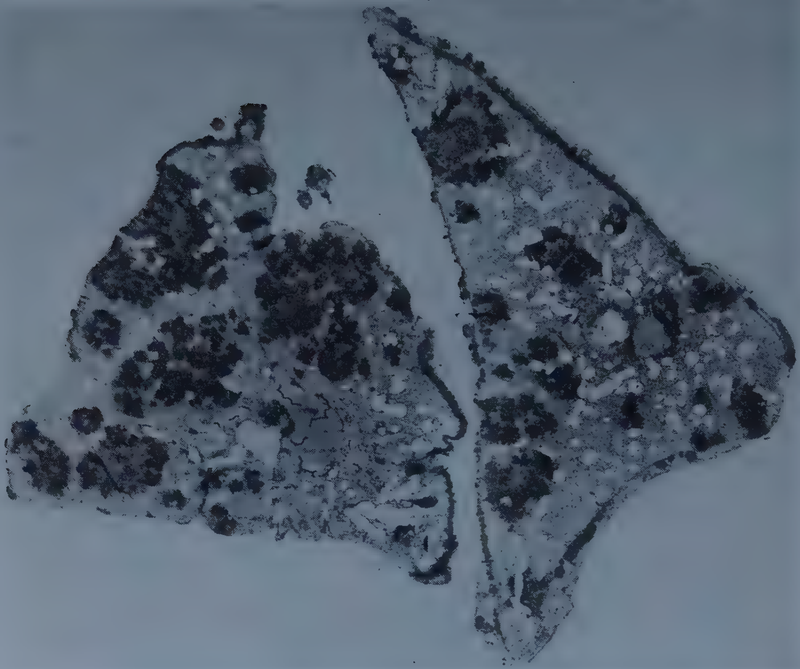


FIGURE 4. Lesions of the lung and pleura of a rat which had received excessive cortisone. Gram-Weigert stain. × 2.

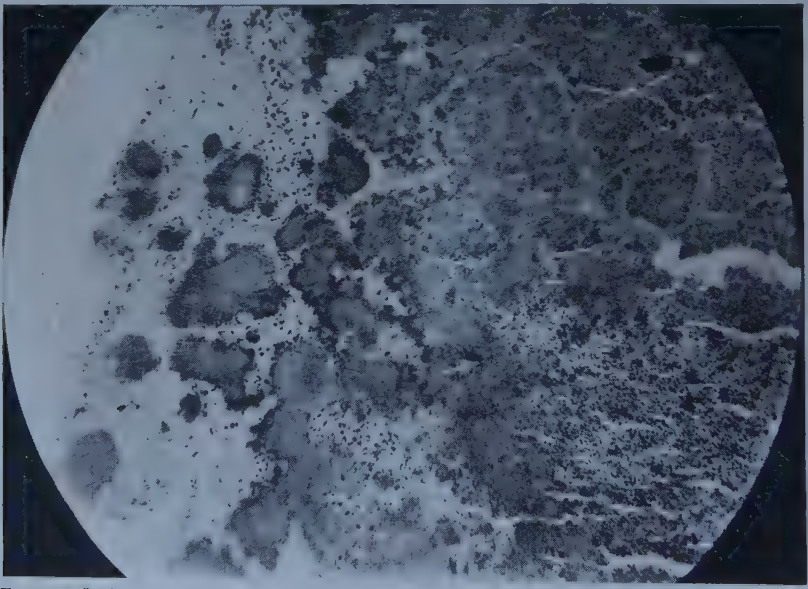


FIGURE 5. Lesion of the lung of a rat which had received excessive cortisone. Note microorganisms grouped as colonies or as individual cells. Gram-Weigert stain. × 10.

culosis during the 28-day period of investigation. In addition, the use of a highly purified agent, oxycellulose corticotoropin,* assayed to be at least 50 times more potent than the commercially available product, failed to produce evidence of pseudotuberculosis.

Continuous administration of corticotropin apparently induces more severe hypercorticalism in the rat than can be caused by intermittent injection of an equal amount of the hormone.[10] In this regard, it should be pointed out that Selye has reported the appearance of a similar systemic infection in piebald rats receiving a daily dose of corticotropin of only 12 mg., which was given at more frequent intervals, every four hours.[11] It is conceivable that this apparently different action of cortisone and corticotropin is merely a reflection of our present inability to judge comparable quantities of the respective drugs and the frequency of administrations in terms of the physiologic effects produced.

In summary, the nature of the results obtained in a particular host appear to depend to a considerable extent upon the precise details of the experiment. Thus, factors in the design of these investigations, including the dosage and duration of hormone administration, the nature of the lesion under evaluation, and the specific host parasite relationship involved, must be considered in the evaluation of results.

It seems clear that the effects of these agents are mediated entirely through host mechanisms. In man, cortisone and corticotropin produce a temporary reduction in the signs and symptoms of tuberculous illness.

In animals with experimental tuberculosis, cortisone has produced a wide variety of striking effects. In mice, cortisone can convert a chronic smoldering tuberculosis into an acute progressive process. This is also true for certain strains of rats infected with human-type tubercle bacilli. In addition, tuberculous lesions in guinea pigs under the influence of cortisone are apparently less-well localized in the initial stages of development.

In contrast, corticotropin has failed to produce evidence of significant alteration in the experimental tuberculosis of mice, rats, and guinea pigs. Another example of the difference in the effects produced has been observed in the regularity of the occurrence of pseudotuberculosis in albino rats under the influence of cortisone. In contrast to the high frequency of pseudotuberculosis in rats given cortisone, no instance of this disease was encountered in a similar group of animals given large quantities of corticotropin.

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THE EFFECT OF CORTISONE AND ACTH ON THE PATHOGENESIS OF TUBERCULOSIS*

By Max B. Lurie, Peter Zappasodi, Arthur M. Dannenberg, Jr.,†
and Eugenia Cardona-Lynch
The Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania

In the study of the role of sex hormones in resistance to infection, it was noted that tuberculosis in rabbits is accompanied by a marked hypertrophy of the adrenal cortex.[1] Furthermore, in subsequent studies, it was found that the weight of the adrenals of the natively most resistant race in our rabbit colony affected by tuberculosis caused by the human-type tubercle bacillus is much greater than that of a susceptible race similarly infected.[2] Therefore, investigations on the role of the adrenal cortex in resistance to the disease were undertaken to determine whether resistance can be increased by increasing adrenal function and, conversely, whether by lowering this function the native resistance can be diminished.

When natively susceptible and resistant rabbits inhale a certain known number of virulent human-type tubercle bacilli, an extensive pulmonary tuberculosis is found, as a rule, five months after infection in the former, and none at all in the latter.[3] If such rabbits, thus quantitatively exposed, are killed five weeks after infection, the number of primary tubercles generated in these two types of animals is inversely proportional to their genetic resistance: the greater the resistance, the fewer the primary pulmonary foci (PLATE 1, FIGURE 1).

Methods and Materials

With the availability of cortisone, the following experiment was performed. Twenty litter mates of the genetically uniform and highly susceptible strain FC were divided into two groups of ten each. Total and differential counts of their blood cells and their fasting blood sugar were determined. At the same time, the spread of India ink and of rabbit hemoglobin in the skin was measured four hours after injection. The inflammation at the site of injection of these substances in the skin was ascertained on the next day. Having obtained these base lines, ten of the rabbits received two mg. of cortisone acetate per kilogram intramuscularly on alternate days. The ten control litter mates received the same volumes of the suspending medium without the cortisone by the same route, at the same intervals. Three days after the beginning of cortisone treatment, when the absolute number of circulating lymphocytes in the blood of the experimental animals had been markedly depressed, and when the fasting blood sugar of the same animals had increased by comparison with the essentially unchanged levels of these items in the control animals, both groups were simultaneously exposed to the quantitative inhalation of known numbers of viable, virulent, human-type tubercle bacilli, H37Rv, in the ap-

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† Charles Hartwell Cocke Memorial Fellow of the National Tuberculosis Association.

paratus for experimental air-borne infection, which has been carefully evaluated as previously described.[4]

After the control and experimental animals were infected, the cortisone and the suspending medium, respectively, were administered to each group at the same intervals and in the same amounts, as stated above, throughout the course of the experiment. During this time, the absolute number of circulating lymphocytes of the blood, the fasting blood sugar, the blood ascorbic acid, the development of tuberculin sensitivity and antibodies against the tubercle bacillus, the spread of India ink and rabbit hemoglobin in the skin, and the inflammation induced by these agents in this tissue were measured. At this time, the excretion of steroids in the urine was also determined.

TABLE 1

THE EFFECT OF CORTISONE ON THE DEVELOPMENT OF THE TUBERCULIN REACTION IN THE SKIN AT DIFFERENT INTERVALS FOLLOWING THE INHALATION OF HUMAN-TYPE TUBERCLE BACILLI

Rabbit number		Inflammation 16–18 days after infection, cu.mm.		Inflammation 21 days after infection, cu.mm.		Inflammation 25–27 day after infection, cu.mm.	
Nontreated	Treated	Non-treated	Treated	Nontreated	Treated	Nontreated	Treated
C 11	E 1	13	0	288	0	391	14
C 12	E 2	17	0	126	12	175	23
C 13	E 3	7	0	530	26	608	14
C 14	E 4	10	0	216	16	288	27
C 15	E 5	95	0	152	0	315	21
C 16	E 6	6	0	54	0	240	10
C 17	E 7	0	0	18	0	68	14
C 18	E 8	8	0	190	0	373	11
C 19	E 9	11	0	92	11	152	21
C 20	E 10	0	0	15	0	83	0
Mean		17 ± 8	0 P = 0.025	168 ± 47	7 ± 3 P = 0.001	269 ± 49	16 ± 2 P = 0.000

Results of Cortisone Treatment

It was found that the absolute number of circulating lymphocytes of the cortisone-treated animals was continuously diminishing, in statistically significant amounts, with continuation of treatment, while that of the controls actually increased, as compared with their respective base lines. Likewise, the fasting blood sugar of the experimental animals increased with the duration of treatment while, in the controls, this increment was much less.

The spread of India ink or hemoglobin in the skin was not definitely affected in either the control or the experimental animals. The inflammation induced by these agents in the skin was markedly reduced in the experimental as compared with control animals, however, and in statistically significant amounts. Likewise, tuberculin produced much more inflammation in the control than in the cortisone rabbits (TABLE 1). This is clearly due to the protective effect exercised by cortisone on the capillaries against agents that increase their permeability, as observed by Menkin. Peptone, which releases fibrinolysin *in vitro*, increases the permeability of the capillaries of normal rabbits to a much greater extent than it does in cortisone-treated animals, (PLATE 1, FIGURES 2a & 2b). Moreover, pure fibrinolysin, a substance released at sites of inflammation, causes marked leakage of the vessels of normal rabbits and scarcely affects cortisone-treated animals, when injected into the skin. Furthermore, the capillary fragility of cortisone-treated rabbits is much less than that of untreated controls, as indicated by capillary hemorrhage induced by applying a uniform suction to the skin, (PLATE 1, FIGURES 3a & 3b). These observations would indicate that cortisone not only protects the capillary wall against chemical agents that disrupt its integrity, but that the tensile strength of this wall against the internal blood pressure is also augmented by the hormone. Both these effects may be the result of physical changes in the ground substance induced by the hormone.[5]

The antibody production against the tubercle bacillus, as revealed by the Middlebrook and Dubos hemagglutination test, was not conspicuously affected, although the titer in the experimentals was slightly though uniformly lower than in the controls.

In view of the demonstration by Gordon and Katsh[6] that starvation, by stimulating the adrenal function, increases the phagocytic activity of the macrophages, half of the control and experimental rabbits were given 6 cc. of a 1:3 dilution of India ink in saline per kilogram intravenously, three hours before they were killed by air embolism on the 34th and 36th day after infection. Another group of three cortisone-treated and three untreated rabbits were similarly injected and killed two weeks after infection. Their livers and spleens were digested with concentrated KOH, and the weight of carbon present in them was determined. Histological preparations of these organs were also studied. It was found that the phagocytosis of carbon particles was markedly increased in the cortisone-treated animals, (PLATE 2, FIGURES 4 and 5). Similarly, tubercle bacilli injected intravenously were more completely removed from the circulation of the treated rabbits, within five minutes after their introduction, than from that of the controls, and the number of bacilli retained in the spleens of the former was correspondingly greater (TABLE 2). The remaining rabbits were killed 35 or 38 days after inhalation. In all cases, each cortisone-treated rabbit was killed on the same day following infection as its control litter mate that had inhaled the same infected air, for the same time, at the same sitting. All animals were starved for 17 hours before they were killed. The glycogen present in the livers of all control and experimental animals was determined, and the weights of the liver, spleen, adrenals, gonads, and pituitary were ascertained.

It was found that the livers of the cortisone-treated rabbits were twice as heavy as those of the controls. This was due to excessive deposits of glycogen and fat in the markedly enlarged liver cells of the experimental animals, obviously due to the physiological effects of the cortisone. The spleens of the cortisone rabbits were markedly reduced in weight, as a result of the lympholytic effect of cortisone, demonstrable even two weeks after infection.[2] The adrenals were markedly atrophied in the experimentals, clearly due to the suppression of the secretion of ACTH by the pituitary, produced by the high level of cortisone in their blood. The thyroids of the experimental rabbits were uniformly, though only moderately, reduced in weight.

It is clear from these results that the experimental animals were under the intense pharmacologic effects of cortisone and that this hormone had modified the remaining functions of the markedly atrophied fascicular and reticular zones of the adrenal cortex as well as the functions of other glands of internal secretion.

The number and size of the tubercles developed in the lungs of the control and experimental rabbits were accurately determined. The number of tubercles generated in the cortisone rabbits was uniformly greater than in the controls. On the average, three to four times as many tubercles resulted from the inhalation of human tubercle bacilli by cortisone-treated rabbits as from the inhalation of the same numbers of bacilli by untreated litter mates of the same inbred strain and the same genetic resistance to the infection. This difference is statistically significant (PLATE 2, FIGURE 6).

TABLE 2

NUMBER OF TUBERCLE BACILLI IN THE BLOOD AND SPLEEN OF NORMAL AND CORTISONE-TREATED RABBITS AT DIFFERENT INTERVALS FOLLOWING THE INTRAVENOUS INOCULATION OF THE SAME SUSPENSION OF H37RV IN UNIT VOLUMES PER KILOGRAM OF BODY WEIGHT

Rabbit number		Blood, no. of bacilli per cc.				Spleen, no. of bacilli per mg.	
		5 minutes after injection		22 hours after injection		22 hours after injection	
Normal	Treated	Normal	Treated	Normal	Treated	Normal	Treated
A 12-12	A 10-3	260,000	157,000	140	1,300	2,200	5,600
A 9-142	A 9-132	183,000	183,000	1,200	130	2,400	3,000
A 12-13	A 12-19	280,000	86,000	486	2,400	4,200	5,500
A 12-14	A 12-29	15,000	7,600	31	110	277	658
A 13-8	A 13-9	16,000	8,500	18	11	200	272
Average		150,800	88,420	375	790	1,855	3,006

The size of the tubercles in the lung, however was uniformly and markedly reduced in the cortisone-treated animals. In the untreated rabbits, the yellow-gray caseous center was surrounded by a wide ring of grayish translucent infiltration; while in the experimental litter mates, on the other hand, there was scarcely any infiltration about the almost-white caseous foci situated nakedly in the unaffected lung parenchyma. Furthermore, the spread of the disease to the draining tracheobronchial lymph nodes and to the internal organs was markedly reduced in the experimental rabbits.

It was found in these experiments as well as in others not detailed here that, initially, notwithstanding the demonstrated greater *in vivo* phagocytic capacity afforded the reticuloendothelial cells by cortisone, the number of inhaled bacilli arrested in the lung of the experimental rabbits was no greater than that retained by the controls.

Two weeks after infection, the bacilli were more numerous in the lungs of the cortisone rabbits as observed both histologically and by culture, (PLATE 2, FIGURES 7 and 8.) In the draining tracheobronchial lymph nodes of the controls, however, they greatly outnumbered those of the experimental animals (TABLE 3).

Five weeks after exposure, the pulmonary lesions in the cortisone rabbits were sharply delimited foci of caseous pneumonia with intra-alveolar plugs of partially caseated cells still retaining their pyknotic nuclei and swarming with inordinate numbers of bacilli. There was little interstitial or perifocal inflammation. These caseous plugs were largely out of contact with the lung parenchyma and its blood and lymph circulation (PLATE 3, FIGURES 9 and 10). The lesions in the controls were tuberculous granulomas with well-advanced, discrete caseous centers from which all cellular boundaries and nuclei had disappeared and which contained moderate numbers of bacilli surrounded by widely spreading zones of infiltrating perifocal inflammation, penetrated by ingrowing capillaries (PLATE 3, FIGURES 11 and 12). These important differences in the histologic character of the lesions in the two types of animals accord well with the gross differences between them described above. Thus, though the bacilli in the pulmonary lesions of the untreated animals were far fewer than in the experimental rabbits, invasion of the blood and lymph by the bacilli occurred more frequently in the former than in the latter (PLATE 4, FIGURES 13 and 14). Similar observations have been made in a more resistant strain of rabbits, AD, exposed to bovine bacilli of reduced virulence.

TABLE 3

NUMBER OF TUBERCLE BACILLI CULTURED FROM 2 MG. OF LUNG AND FROM DRAINING TRACHEOBRONCHIAL NODES OF CONTROL AND CORTISONE-TREATED RABBITS TWO WEEKS AFTER SIMULTANEOUS INHALATION OF HUMAN-TYPE TUBERCLE BACILLI

Rabbit number		Lungs		Tracheobronchial nodes	
Control	Experimental	Control	Experimental	Control	Experimental
Ca 5-10	Ca 5-9	103	877	21	2
Ca 5-21	Ca 5-20	191	360	30	9
Ca 5-19	Ca 5-47	224	305	68	0.7

Much of the difference in the pathogenesis of tuberculosis in the two types of animals may be understood on the basis of the two observed effects of the hormone: (1) Pharmacologic doses of cortisone markedly interfere with the digestive capacity of the macrophages for the ingested bacilli. (2) Cortisone, by virtue of the protection it affords the capillaries against agents that increase capillary permeability and, hence, its antiphlogistic effect conspicuously influences the character of the lesion, its extent, and its dissemination in the body. The former explains the genesis of greater numbers of primary tubercles occurring in the treated animals. It must be clearly understood that present evidence[3] indicates that the majority of human-type tubercle bacilli ingested by the alveolar phagocytes of even a very susceptible rabbit are soon destroyed and do not accumulate sufficiently within these cells to generate a grossly visible tubercle. Cortisone, by interfering with the digestive capacity of these cells, allows many more bacilli to multiply sufficiently within the alveolar phagocytes to yield visible primary foci. The antiphlogistic influence of the hormone explains not only the suppression of the perifocal inflammation about the intra-alveolar plugs swarming with tubercle bacilli in the cortisone rabbits and the marked reduction of the tuberculin reaction in these rabbits, but also the partial localization of the disease at the portal of entry, for the suppression of the inflammation retards the ingrowth of capillaries and lymphatics into the isolated alveolar plugs where the bacilli swarm. Hence, withdrawal of cortisone reverts the sensitivity of the capillary walls to agents that increase permeability to its original state, with the consequent intensification of the tuberculin reaction and the development of an intense perifocal inflammation about the accumulated masses of bacilli in the lungs that had resulted from the previous hormone treatment (PLATE 4, FIGURE 15). This led to massive consolidating pulmonary tuberculosis with liquefaction, rupture into the bronchi, hematogenous dissemination and a fatal issue in natively susceptible rabbits, and to progressive disease with cavity formation in natively resistant rabbits. At this time, the lesions in the simultaneously infected untreated rabbits were rapidly regressing in the susceptible rabbits and had completely healed in most of the resistant animals.[2]

No evidence has thus far been obtained to indicate that the inordinate accumulation of the tubercle bacilli in the cells of the cortisone-treated rabbits can be accounted for by a stimulating effect of the hormone on the growth of the bacilli. Cortisone, in the concentration present in the body of the experimental animals, did not enhance the growth of tubercle bacilli *in vitro*. The extracellular multiplication of the bacilli *in vivo* was markedly suppressed when bacilli within collodion-coated silk bags were placed into the peritoneal cavity of hormone-treated rabbits. Yet, one of the most significant effects of pharmacologic doses of cortisone in tuberculosis is its profound influence on the physiology of the macrophages. While the hormone affords these cells increased phagocytic activity, their digestive capacity for the ingested bacilli is markedly depressed.

The Effect of ACTH

It was noted above that the pharmacologic doses of cortisone used in these experiments caused marked atrophy of the fascicular and reticular zones of the adrenal cortex and, hence, necessarily modified their functions. Since tuberculosis in resistant races is associated with hypertrophy of the adrenal cortex, it is possible that physiologic rather than pharmacologic doses of these and other adrenocortical and hypophyseal hormones may actually enhance the defense mechanisms against infection.

To determine what effect physiological stimulation of the adrenal cortex has on resistance to tuberculosis, two susceptible strains of rabbits were chosen, one, the FC race reported above, and the other, the C strain. The latter differs from the FC strain in that its adrenals are genetically much larger than those of the former. Both strains were divided into two groups. The first group received daily intramuscular injections of sheep ACTH in gelatin for 3 days, 0.5 mg. per kilo. The control litter mates received saline by the same route. Both groups were then simultaneously exposed to the quantitative inhalation

TABLE 4

THE EFFECT OF 0.5 MG. OF SHEEP ACTH IN GELATIN DAILY PER KILO OF BODY WEIGHT ON THE NUMBER OF PRIMARY PULMONARY TUBERCLES GENERATED BY THE QUANTITATIVE INHALATION OF H37RV IN THE SUSCEPTIBLE FAMILIES FC AND C, RESPECTIVELY

Rabbit number	Weight of adrenals mg./100 g.	Weight of liver g./100 g.	Number of bacilli inhaled estimated	Number of tubercles generated	No. of bacilli yielding one tubercle	Rabbit number	Weight of adrenals mg./100 g.	Weight of liver g./100 g.	Number of bacilli inhaled estimated	Number of tubercles generated	No. of bacilli yielding one tubercle
FC family, control						*FC family, treated*					
FC 4-9	12.8	2.1	20,618	671	31	FC 4-18	12.7	2.3	41,715	486	86
FC 2-48	6.4	2.2	53,821	1,442	37	FC 4-21	18.9	2.6	20,935	223	94
FC 4-24	17.1	2.4	20,935	341	61	FC 2-52	14.4	2.2	47,390	477	99
FC 4-17	7.9	1.7	45,237	632	71	FC 4-16	12.1	2.1	44,125	357	124
FC 4-30	16.0	2.1	38,748	345	112	FC 4-15	15.2	2.6	20,776	140	148
Mean	12.0 ± 1.9	2.1 ± 0.11			62 ± 14*	Mean	14.7 ± 1.1	2.3 ± 0.10			110 ± 11*
C family, control						*C family, treated*					
C 12-16	22.3	2.2	45,359	1,187	38	C 12-12	26.0	4.1	47,051	1,672	28
C 12-20	28.7	2.4	13,062	313	42	C 12-24	24.1	3.1	11,755	297	40
C 12-1	28.0	2.3	35,411	833	43	C 11-20	30.9	2.8	36,709	789	47
C 12-18	23.0	2.8	12,502	262	48	C 12-27	21.9	3.2	20,142	308	65
C 12-28	20.6	2.8	21,252	323	66	C 12-17	26.6	3.2	13,248	190	70
Mean	24.5 ± 1.4	2.5 ± 0.13‡			47 ± 4†	Mean	25.9 ± 1.3	3.3 ± 0.18‡			50 ± 7†

* C. R. of the difference between these figures is 2.7 with "P" value of 0.01.
† The difference between these two figures is not significant.
‡ C. R. of the difference between these figures is 3.6 with a "P" value of 0.004.

TABLE 5

THE EFFECT OF 0.5 MG. OF PORCINE ACTH IN GELATIN DAILY PER KILO OF BODY WEIGHT ON THE NUMBER AND SIZE OF PRIMARY PULMONARY TUBERCLES GENERATED BY THE QUANTITATIVE INHALATION OF H37RV IN THE SUSCEPTIBLE FAMILY FC

Rabbit number	Weight of adrenals, mg./100 g.	Weight of liver, g./100 g.	No. of bacilli inhaled (estimated)	No. of tubercles generated	No. of bacilli yielding one tubercle	Average diameter of tubercle, mm.	Rabbit number	Weight of adrenals, mg./100 g.	Weight of liver g./100 g.	No. of bacilli inhaled (estimated)	No. of tubercles generated	No. of bacilli yielding one tubercle	Average diameter of tubercle, mm.
Control							*Treated*						
FC 4-13	17	1.6	23,866	635	38	5.0	FC 4-14	13	3.1	23,694	1,350	18	3.0
FC 4-4	17	1.6	26,270	530	49	4.4	FC 5-6	8	2.2	22,606	517	44	2.5
FC 5-7	13	2.2	21,865	440	50	3.5	FC 4-32	11	2.7	28,165	571	49	3.0
FC 4-29	12	2.5	25,015	359	70	3.1	FC 5-2	16	2.0	21,865	401	54	3.9
FC 6-8	16	2.2	33,998	445	76	3.6	FC 4-31	14	2.5	24,553	434	56	2.4
FC 4-33	11	1.9	27,980	330	85	3.2	FC 6-3	29	1.9	37,998	605	63	3.3
FC 6-1	16	1.9	37,141	408	93	3.0	FC 6-7	18	2.3	34,569	391	88	2.5
FC 4-26	10	1.7	27,128	273	99	3.0	FC 4-11	19	3.7	23,866	125	191	2.8
FC 5-11	7.5	1.9	36,855	209	176	4.5							
Mean	13.3 ± 1.2	1.9 ± 0.1*			82 ± 14	3.7 ± 0.23†		16 ± 2.3	2.5 ± 0.2*			70 ± 19	2.9 ± 0.18†

* The "P" value of the difference between these observation is 0.008.
† The "P" value of the difference between these observation is 0.007.

of human-type tubercle bacilli. The ACTH and the saline treatment, respectively, were continued throughout the experiment. The only evidence of a possible physiological effect of the ACTH in the FC rabbits was a constant polymorphonuclear leukocytosis. While the lymphocytes tended to be suppressed by the hormone, this effect was not uniform. In the FC race neither the weight of the liver nor its glycogen content was affected by this ACTH preparation. In the C race, however, the same preparation in the same amounts caused a statistically significant increase in liver weight and a definite increment in the glycogen deposited in the liver (TABLE 4). In other words, the sheep ACTH used contained an insufficient amount of corticotropin to stimulate an excess secretion of physiologically detectable cortisonelike steroids from the small adrenals of the FC family but enough to cause an excess of physiologically detectable steroid from the genetically large adrenals of the C family. It was, therefore, of interest to find that this ACTH treatment increased the resistance of the FC rabbits and reduced the number of tubercles generated by about one half (TABLE 4 and PLATE 4, FIGURE 16). It had no effect on the C rabbits. This would suggest that the beneficial effect of the ACTH treatment in the FC rabbits is not due to an excess secretion of glucocorticoids.

In another experiment (TABLE 5), the same FC strain, with its genetically small adrenals, was treated with the same dose of a porcine ACTH in gelatin. This ACTH preparation caused a marked increase in liver glycogen and a significant increment in liver weight, thus eliciting an excess secretion of oxycorticosteroids. It is noteworthy, therefore, that this corticotropin simulated to some extent the effect of cortisone on the pathogenesis of the disease by slightly increasing the number of tubercles generated and reducing their size.

Summary

Cortisone affects in a fundamental fashion all the essential mechanisms involved in the pathogenesis of tuberculosis. It increases the accumulation of tubercle bacilli within the macrophages though their phagocytic activity is enhanced. It suppresses nonspecific and allergic inflammation as a result of the protective effect exerted by the hormone against many agents that increase capillary permeability. This antiphlogistic influence, by suppressing the migration of cells to and from the tubercles, and by reducing the ingrowth of capillaries into these foci, interrupts the bridge between the lesion and the rest of the body and tends to isolate it partially and to localize it temporarily and incompletely at the portal of entry. The reduced capillary permeability and the lympholytic effect of the hormone may also be instrumental in the retardation of the development of the caseous process and the diminution of antibody production, which is characteristic of the cortisone-treated animals; for allergic sensitization, of which caseation is a part, has now been demonstrated to be at least partly mediated by antibodies.[7] Lymphocytes are probably concerned in antibody production,[8] and the transport of antigens and antibodies is influenced by inflammation. Studies directed to elucidate the effect of cortisone on the intercellular ground substance, on cellular permeability, and on the enzyme functions of the phagocytes are most desirable.

Thus, in tuberculosis, cortisone acts like a double-edged sword. The very suppression of the inflammation, while it tends to isolate the infection from the rest of the body, nevertheless permits the local multiplication of the bacteria, for the phagocytes and the humoral agents concerned with combatting the infection arrive tardily and in low concentration. Furthermore, there is general agreement that cortisone administered during the course of immunization tends to lower antibody production, which would also militate against the host. Again, while there is ample evidence that increased adrenal function and cortisone stimulate phagocytosis of particulate matter and bacteria, the digestive capacity of the cells for the ingested microorganisms is markedly reduced, whether these be tubercle bacilli, pneumococci, streptococci, or even red blood cells.[9] Hence, the bacteria can accumulate in the tissues in the absence of symptoms of fever and toxemia, which are suppressed by the hormone.

The significance of the ACTH treatment reported in this study is not quite clear at present. Nevertheless, it would appear that by suitable, sheep ACTH stimulation, the resistance of certain animals whose adrenal function, as indicated by their mass, is low can be increased by corticotropin; whereas similar treatment of rabbits with higher natural adrenal function exercises no effect. Furthermore, even in rabbits with low adrenal function, beneficial effects from ACTH can be obtained only within very narrow limits. A corticotropin of greater potency may induce cortisone effects that nullify the protective influence that may stem from corticotropin. Much further investigation is necessary to establish this interpretation.

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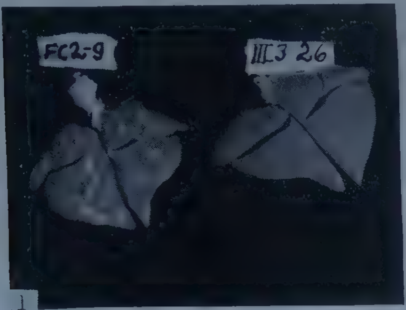

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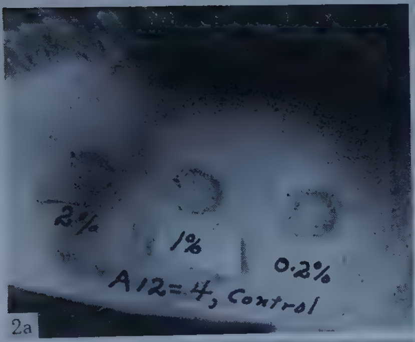

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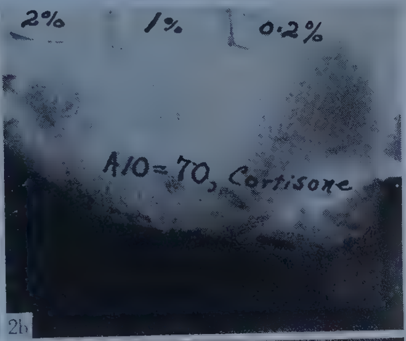

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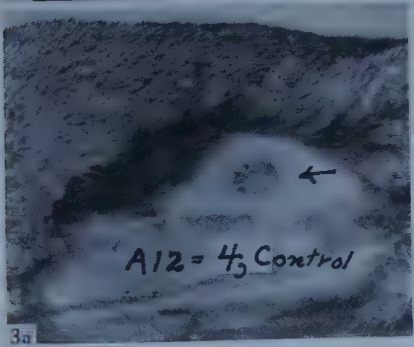

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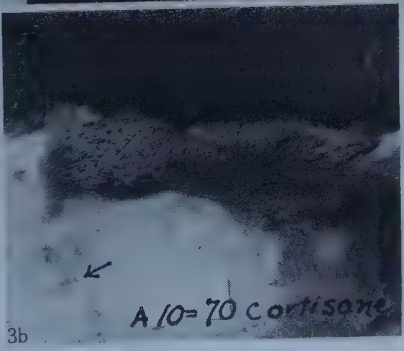

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PLATE 1

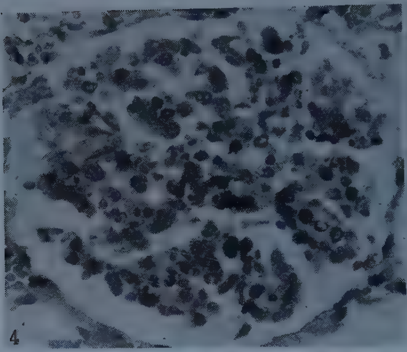
FIGURE 1. The lungs of susceptible rabbit FC 2-9 and of a resistant rabbit III 3-26, 32 days after the simultaneous inhalation of 4200 to 4300 human type tubercle bacilli. One of 39 inhaled human-type bacillary units generated a primary tubercle in the susceptible animal, while only one of 1000 inhaled bacillary units gave rise to a pulmonary focus in the resistant animal.

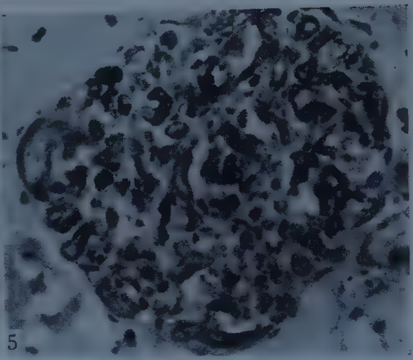
FIGURE 2a. Extravasation of intravenously injected trypan blue at the sites of the intracutaneous injection of 2, 1, and 0.2 per cent peptone, respectively, in the untreated rabbit A 12-4.

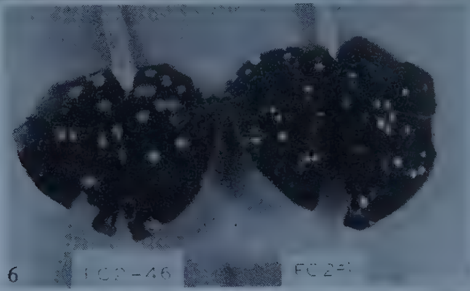
FIGURE 2b. In the cortisone-treated rabbit, A 10-70, the trypan blue extravasated to a considerable extent only at the site of injection of 2 per cent peptone. A trace of the dye left the vessels at the site of injection of 1 per cent of the irritant, while none left the vessels at the site of injection of 0.2 per cent peptone.

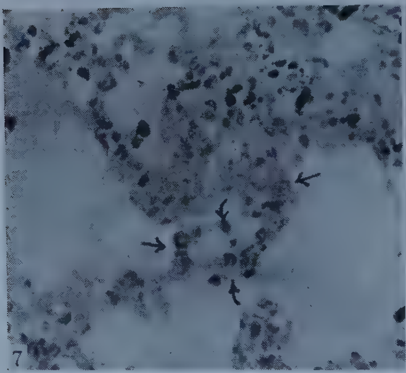
FIGURE 3a. Capillary hemorrhage, indicated by the arrow, at the site of suction in the skin of the normal rabbit, A 12-4.

FIGURE 3b. No capillary hemorrhage in the cortisone-treated rabbit, A 10-70, at the site of application of the same degree of suction for the same time as in the normal rabbit shown in FIGURE 3a. This site is indicated by the ring and arrow.









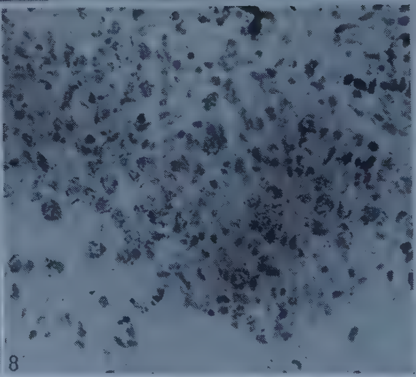


PLATE 2

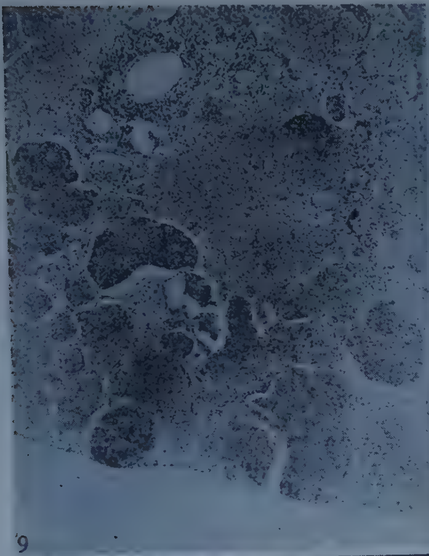
FIGURE 4. Scant phagocytosis of carbon particles by the cells of the kidney glomerulus of the untreated rabbit, Ca 5-10, 18 hours after the intravenous injection of 6 cc. per kilo of a 1:3 dilution of India ink.

FIGURE 5. Intense phagocytosis of carbon particles by the cells of the kidney glomerulus of the cortisone-treated rabbit, Ca 5-20, given the same amount of the same suspension of India ink per kilo and killed at the same interval after the intravenous injection as Ca 5-10 shown in FIGURE 4.

FIGURE 6. The lungs of the untreated rabbit, FC 2-46, and of the cortisone-treated rabbit, FC 2-1, 34 days after the simultaneous quantitative inhalation of human type tubercle bacilli. Both lungs are black from the intravenous injection of India ink. The tubercles in the control rabbit are much fewer than in the treated animal. 81 inhaled bacilli were necessary to generate one pulmonary tubercle in the former, while 39 microorganisms were sufficient for the latter. The size of the tubercles are markedly smaller in the cortisone rabbit, however. In the untreated rabbit, the yellowish-white caseous centers are surrounded by a wide ring of translucent gray infiltration. In the experimental rabbit, there is scarcely any infiltration about the brightly white caseous foci situated nakedly in the unaffected lung parenchyma.

FIGURE 7. One of the rare primary foci in the lung of the untreated rabbit, Ca 5-10, 2 weeks after a quantitative inhalation of human-type tubercle bacilli. The arrows indicate the meager bacilli in this earliest microscopic evidence of the intracellular growth of the microorganism in a control animal.

FIGURE 8. One of the much larger and more common primary foci in the lung of the cortisone-treated rabbit, Ca 5-9, a litter mate of Ca 5-10 shown in FIGURE 7. Both rabbits were killed 2 weeks after a simultaneous quantitative inhalation of human-type bacilli. Numerous intracellular tubercle bacilli are found. There is no lack of phagocytosis. The accumulation of tubercle bacilli in the mononuclears of the cortisone-treated rabbit is far greater than that in the phagocytes of the untreated control animal.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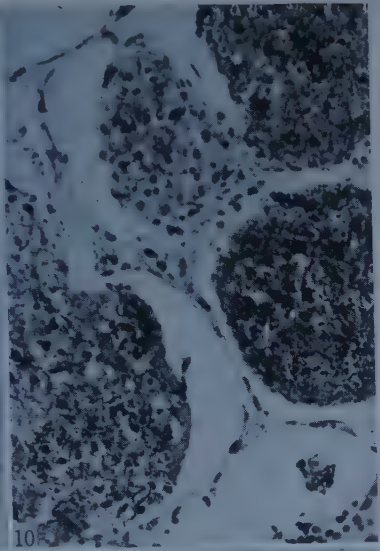
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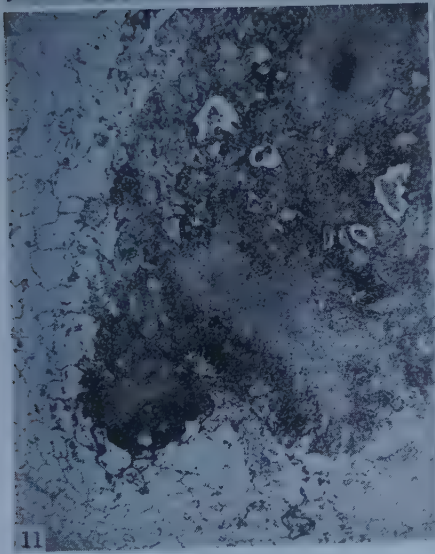
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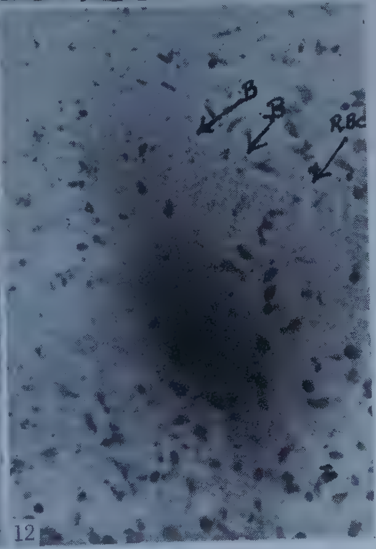

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PLATE 3

FIGURE 9. The lung of the cortisone-treated rabbit, FC 3-39, 35 days after the quantitative inhalation of human bacilli, showing an area of caseous pneumonia with intra-alveolar plugs of partially caseated cells.

FIGURE 10. A higher magnification of the same lung as depicted in FIGURE 9 showing the swarming of inordinate numbers of bacilli in the necrotic alveolar plugs. Note that these plugs are not contiguous with the alveolar septa. These septa are extremely thin and not infiltrated or inflamed.

FIGURE 11. The lung of FC 3-37, an untreated litter mate of rabbit FC 3-39, shown in FIGURES 9 and 10. Both rabbits were killed at the same time after the simultaneous inhalation of human-type bacilli. The lung of the untreated rabbit shows a tuberculous granuloma with well-advanced caseous centers surrounded by a widely spreading zone of infiltrating perifocal inflammation.

FIGURE 12. The lung of FC 2-47, an untreated rabbit, killed 34 days after the quantitative inhalation of human-type bacilli. The periphery of a far-advanced caseous center with complete disappearance of cellular structures is seen. Occasional bacilli are indicated by the arrows marked B. Hemorrhage from a ruptured capillary, indicated by the arrow marked RBC, is in close proximity to the bacilli.

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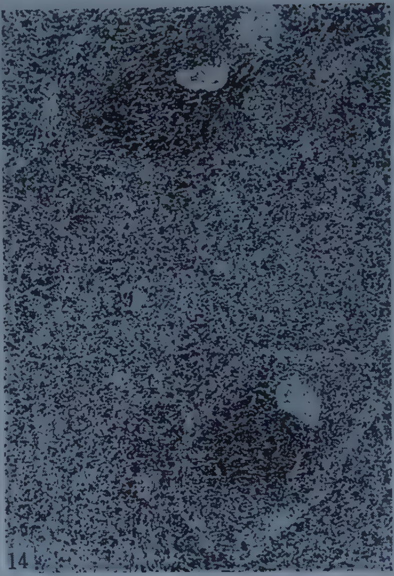

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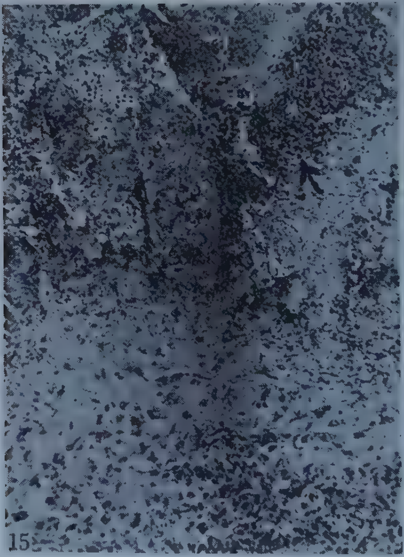

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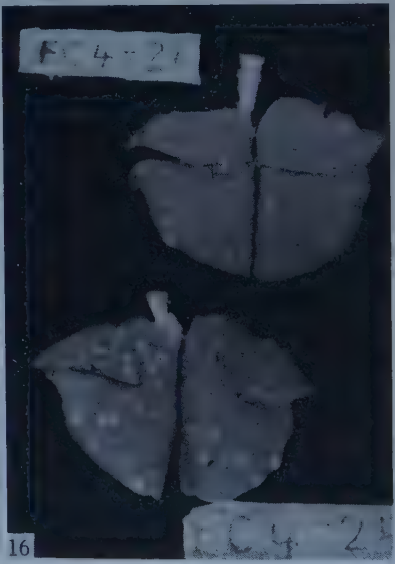

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PLATE 4

FIGURE 13. The spleen of the untreated rabbit, FC 3-29, killed 38 days after the quantitative inhalation of human-type tubercle bacilli. A large hematogenous tubercle is seen.

FIGURE 14. The spleen of the cortisone-treated rabbit, FC 3-30, killed at the same time after the same quantitative inhalation of human-type tubercle bacilli as the control rabbit, FC 3-29, shown in FIGURE 13. There is no hematogenous tuberculosis in this spleen.

FIGURE 15. The lung of rabbit Ca 4-7, which had inhaled about 16,000 tubercle bacilli of the human type. The rabbit was under the influence of cortisone for 45 days. The hormone was then withdrawn and the rabbit died 34 days later of massive caseous pneumonia, liquefaction, and bronchial and hematogenous dissemination. The withdrawal of the hormone induced a severe perifocal inflammation, indicated in the lower half of the photograph, about the caseous plugs swarming with bacilli that had resulted from the previous cortisone-treatment. These caseous plugs are shown at the top of the microphotograph. Areas of liquefaction are shown by the arrow where there is an intense polymorphonuclear infiltration.

FIGURE 16. The lungs of FC 4-21 and its litter mate FC 4-24, both of which had inhaled about 21,000 human type tubercle bacilli, simultaneously. Both rabbits were killed 31 days after exposure. In FC 4-21, treated with sheep ACTH in gelatin, 94 inhaled bacilli yielded one tubercle. In the untreated control rabbit, FC 4-24, 61 inhaled bacilli generated one pulmonary focus.

POLIOMYELITIS INFECTION BY PARENTERAL ROUTES MADE POSSIBLE BY CORTISONE*

By Gregory Shwartzman and Stanley M. Aronson
Department of Microbiology, The Mount Sinai Hospital, New York, N. Y.

It was previously reported that cortisone produces a significant enhancement of intracerebral infection with MEF_1 and Lansing strains of poliomyelitis virus in the Syrian hamster. The enhancement manifests itself in increased severity of symptoms, shortening of the incubation period, marked reduction in survival time, and a high mortality rate. The effect of cortisone is protracted, the duration depending on the dose used. There is a reciprocal relationship between the dose of cortisone and the amount of virus; the larger the dose of the hormone, the smaller the amount of virus necessary to produce the infection. An amount of virus of low infectivity causes a marked disease with a high mortality rate in hamsters receiving a single injection of 3 mg. of cortisone, while a dose as small as 2 mg. gives a highly fatal disease with a stronger concentration of the virus. It is of particular interest that there was a two log increase in the concentration of the virus in the brain of cortisone-treated hamsters. It was also reported that ACTH failed to change the susceptibility of the hamster to the poliomyelitis infection.[1, 2, 3]

In extending these observations it was possible to produce a violent and uniformly fatal poliomyelitis infection in this animal by peripheral routes (*i.e.*, intraperitoneal, intramuscular, and subcutaneous), following treatment with cortisone.

As may be seen from TABLE 1 it was possible to infect hamsters consistently by the intraperitoneal route with the aid of cortisone. The animals developed a rapidly progressing, highly fatal disease, the severity of which equalled and possibly exceeded that obtained on intracerebral inoculation of the virus into cortisone-treated hamsters. The results were unequivocal, inasmuch as no clinical disease was elicited following the inoculation of the virus into hamsters receiving no cortisone. There was a clear-cut reciprocal relationship between the amount of cortisone and the concentration of the virus necessary for the production of the disease. The mean survival time and the mortality rate were readily influenced by these relationships, resulting under optimal conditions in an incubation period as short as 3-5 days and a 100 per cent mortality rate. It is of interest that a single injection of 5 mg. was less effective than two injections of 2 mg. given at an interval of 24 hours. Four injections of 2 mg. gave rise to a fatal disease after the inoculation of 0.5 ml. of the virus diluted as high as 1:8000. Even after taking into consideration the fact that a volume ten times greater was employed for the intraperitoneal than for the intracerebral inoculation, it may still be assumed that the intraperitoneal route compares quite favorably with the intracerebral route in cortisone-treated hamsters.

The identity of the disease was established as follows: (1) There was ob-

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* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

tained a typical clinical disease in a great majority of animals, frequently resulting in tetraplegia in animals surviving for at least four days. Characteristic histological changes were invariably seen in the spinal cord in the animals showing the disease during life. (2) The virus recovered from the brain of the intraperitoneally infected hamsters produced typical symptoms in mice. It was neutralized by the anti-Lansing convalescent monkey serum on intracerebral testing in mice. In these titrations 200 mouse LD$_{50}$ were completely neutralized by the serum diluted 1:10, following incubation of the mixture for one hour in a water bath at 37°C. (3) It was also possible to neutralize the virus in the hamsters. In these experiments, the hamsters received four injections of 2 mg. of cortisone at intervals of 3, 20, and 3 hours. One-half ml. of suitable dilutions of virus and serum incubated in a water bath at 37°C. for one hour were injected intraperitoneally immediately following the fourth injection of cortisone. There was obtained consistent neutralization of the virus diluted 1:500 with the serum diluted 1:40; while, in the absence of the serum, the virus similarly diluted and incubated gave 100 per cent mortaility with a mean survival time of five days.

Table 1

MEF$_1$ Infection in Hamsters by the Intraperitoneal Route

Total no.	Treatment with cortisone	MEF$_1$		Paralysis onset, range of days	Mean survival time	Mortality rate, %
		Time of inoculation	Inoculum dilution			
121	—	—	1:20 to 1:12000		—	0
45	2 mg. 4×, 24, 27, 20 hr.	—	—	—	14	6
10	2 mg. 1×	2 hr. after cortisone	1:20	5–6	7.3	30
10	5 mg. 1×	Immed. after cortisone	1:20	6–16	12	60
10	2 mg. 2×, 24 hr.	2 hr. after 2nd cortisone	1:20	5–8	7.4	100
10	2 mg. 3×, 19, 27 hr.	Immed. after 3rd cortisone	1:1000	4–10	8	66.7
10	2 mg. 4×, 24, 27, 20 hr.	"	1:100	3–5	4.7	100
15		"	1:500	5–10	8	100
10		"	1:1000	5–9	7.6	55.5
10		"	1:8000	5–12	9	60
10		"	1:12000	4–11	—	0
10	3 mg. 3×, 19, 24 hr.	2½ hr. after 3rd cortisone	1:20	4–7	6.5	100
10	3 mg. 4×, 4, 20, 3 hr.	Immed. after 3rd cortisone	1:100	3–5	5.5	100

Hamsters treated with cortisone and receiving the virus intraperitoneally developed a pronounced viremia, apparently due to the multiplication of the virus outside of the central nervous system, as shown in TABLE 2. The observations by Bodian[4] and Horstmann[5] on viremia in chimpanzees infected *per os* are of special interest in this connection.

Daily determinations of the concentration of the virus in various organs were then undertaken to find out the progression of the virus from the site of the inoculation. It may be concluded from the data summarized in TABLE 3 that, during the first 24 hours, the virus appeared in the concentration of two logs in the periadrenal fat, which may well be the amount of virus injected. The virus rapidly multiplied in this site to an ultimate concentration as high as 4.9 logs, however. The next site of active multiplication are the paraverte-

TABLE 2

RECOVERY OF VIRUS FROM HAMSTERS INFECTED INTRAPERITONEALLY

Treatment with cortisone	MEF₁ inoculum dilution	Organ tested			Result in mice†
		Name	Hr after inoculation	Dilution	
—	1:20	Brain	96	1:20	0/8
5 mg. 1×	1:20		20	1:20	0/7
	1:20		70	1:20	0/8
	1:20		192	1:20	5/8
—	1:20	Serum	96	1:2	0/8
5 mg. 1×	1:20		70	1:10	0/6
	1:20		192	1:10	0/6
2 mg. 2×, 24 hr.	1:20		23	1:10	1/16
	1:20		23	1:100	0/16
	1:20		96	1:10	13/16
	1:20		96	1:100	2/16
3 mg. 3×, 19, 24 hr.	1:20	Brain	96	1:100*	6/6
	1:20	Serum	96	1:10	11/16
	1:20		96	1:100	4/16
3 mg. 4×, 4, 20, 3 hr.	1:100	Brain	120	1:100	6/6
	1:100		120	1:1000	5/6
	1:100		120	1:5000*	5/6
	1:100	Serum	120	1:10	6/6
	1:100		120	1:100	2/6
	1:100		120	1:1000	1/6
5 mg. 4×, 19, 24, 24 hr.	1:1000		72	1:10	6/6
	1:1000		72	1:100	3/6
	1:1000		72	1:1000	0/6
	1:1000		120	1:10	6/6
	1:1000		120	1:100	6/6
	1 1000		120	1:1000	5/6
	1:1000		120	1:4000	1/6

*Higher dilutions not tested.
† Numerator = No. of dead animals; denominator = total no. of animals tested.

TABLE 3

PROGRESSION OF VIRUS IN CORTISONE TREATED HAMSTERS FOLLOWING INTRAPERITONEAL INOCULATION

Day	Periadrenal fat	Paravertebral muscle	Spinal cord	Limb muscle
1	2.0*	0	0	0
2	3.78	3.69	0	0
3	4.77	4.3	0	0
4	4.9	4.3	3.78	<1
5	<4.9	4.7	3.78	3.3

Mesenteric tissue, 0.
Lung tissue, always less than 1.
* Numbers express minus log mouse LD_{50}.

bral muscles. While the spinal cord contained no virus during the initial three days, there occurs a sudden multiplication on the fourth and fifth days to a final concentration of 3.78 logs incidentally to paralysis. Little or no virus can be detected in the mesentery, mesenteric lymph nodes, and lungs. In the foreleg muscles, the virus does not appear until the fifth day. Although speculative, it seems that the late appearance of the virus in these muscles may be due to egress from the central nervous system, since the multiplication takes place at a high concentration of the virus in the spinal cord.

Thus, it may be concluded that the periadrenal fat is the initial site of multiplication of the virus following the intraperitoneal inoculation into cortisone-treated hamsters. The virus seems then to spread from the paravertebral muscles to the spinal cord. It is worthy of special emphasis that the multiplication of the virus in the spinal cord occurs after a lag period of three days and at the time when a high concentration of the virus has already been reached in the periadrenal fat and the paravertebral muscles. It is highly suggestive, therefore, that an extraneural concentration of the virus in the vicinity of the peripheral route of inoculation may be a phase essential for the invasion of the central nervous system. Since the peritoneal fluid, mesentery, and mesenteric lymph-nodes remained free of virus, it may be assumed that there are selective sites for extraneural multiplication, preference being shown for the periadrenal fat on intraperitoneal inoculation.

The correlative histological studies were carried out in conjunction with the above experiments with parallel groups of hamsters sacrified at daily intervals.

The initial microscopic changes were observed in the spinal cord, usually the lumbar and cervical enlargements. These alterations, consisting of chromatolysis and acute necrosis of the anterior horn neurons, were noted about four days following intraperitoneal inoculation of the virus. Twenty-four to 48 hours later a belated and sparse inflammatory reaction was seen in the vicinity of these foci. Approximately 36–48 hours following the inception of demonstrable myelitis, a series of degenerative changes were visible in the paravertebral muscles. In a small proportion of animals, the localized myopathy was coincident with the spinal cord alterations, but never preceded it. The first unequivocal evidence of poliomyelitis infection consisted of fiber separation, fusiform swelling, segmental loss of striation and hyaline degeneration (FIGURE 1). The cytoplasm showing hyaline degeneration was homogeneous and glassy. Various degrees of eosinophilia were observed at this stage, being rapidly displaced by irregular zones of basophilic discoloration. As yet, the structural integrity of the muscle fiber was maintained, although some transverse fracturing was apparent. A granular necrosis rapidly supervened, the outline of the muscle cell was often inapparent and a minimal inflammatory cell invasion commenced (FIGURE 2). Specimens derived at a later stage revealed widespread fiber necrosis in association with a restrained inflammatory response and sarcolemmal cell proliferation (FIGURE 3). The histopathological pattern was identical in most respects with skeletal myositis caused by the Coxsackie virus. The deeply basophilic granules in foci of intense muscle necrosis had the staining characteristics of calcium (FIGURE 4). The myositis developed fully within 48 hours. No regenerative features were demonstrable within the limits of survival (six days). Skeletal muscles distal to the vertebral column remained unchanged during the period of observation. The changes within the periadrenal and retroperitoneal adipose tissues consisted essentially of intense necrosis, calcification, and secondary inflammatory cell exudation, and commenced about the same time as the paravertebral myositis.

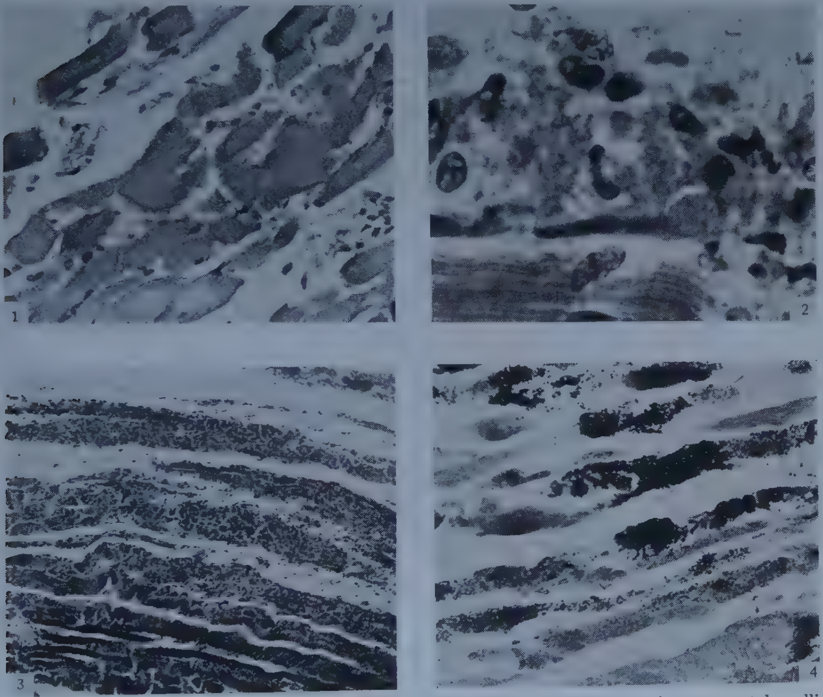

FIGURE 1. Paravertebral muscle showing initial changes consisting of fiber separation, segmental swelling, patchy loss of striations and hyalin degeneration. No inflammatory reaction is apparent. Cortisone-treated hamster infected intraperitoneally. Stain, H & E. 100×

FIGURE 2. Skeletal muscle demonstrating a later stage of myositis. There is total granular necrosis of the involved segment, with invasion of inflammatory elements. Stain, Phosphotungstic Acid-Hematoxylin. 1300×

FIGURE 3. Low power view of late stage of myositis. Most fibers are involved and a perceptible inflammatory reaction is present. Note the close resemblance to Coxsackie virus myositis. Stain, H & E. 100×

FIGURE 4. Field similar to that seen in FIGURE 3 but stained by von Kossa technique. The black precipitate indicates presumptive calcific deposition The presence of calcium was proven by a microchemical crystallization test. 100×

A discrepancy appears to exist between the viral and morphological studies. The initial histopathological changes were found in the spinal cord followed subsequently by lesions in fat and paravertebral muscles. The virus titrations indicated a reversed sequence of localization. It may be assumed that each tissue responds differently to viral invasion. Thus, motor neurons show typical neural cytolysis within 24 hours after invasion by poliomyelitis virus. In contrast, periadrenal fat and paravertebral muscles do not undergo any evidence of damage during two or three days of active viral proliferation. A variable latency in the development of the lesions could account for the diversity of results in viral and histopathological studies.

Cortisone also enhances markedly the disease in the monkey and increases the incidence of takes in peripheral routes. There is an acceleration of infection in the adult mouse and a high rate of takes in the suckling mouse.

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CORTISONE AND INFECTION*

By Lewis Thomas
Pediatric Research Laboratories of the Heart Hospital, University of Minnesota, Minneapolis, Minnesota

Cortisone has the property of completely disorganizing the natural host–parasite relationship, with the outcome overwhelmingly in favor of the parasite, and infection is thereby enhanced. This has been shown to be the case with many unrelated infecting agents and in many unrelated hosts.[1] The list of agents continues to expand: at the moment it includes pneumococci,[2] tubercle bacilli,[3] streptococci,[4, 5] staphylococci,[1] brucellae,[6] typhoid bacilli,[3] spirochaetes,[8] trypanosomes,[9] *Corynebacteriae*,[10] malaria parasites,[11] several varieties of fungi,[12] and the viruses of poliomyelitis,[13] influenza,[14] Coxsackie disease,[15] and rabbit fibroma.[16] The hosts in which the enhancing effect of cortisone has been demonstrated include mice, rats, hamsters, guinea pigs, rabbits, monkeys, human beings, and chick embryos. As biological phenomena go, especially phenomena that can be manipulated in the laboratory, this one has large implications.

One implication has to do with the problem of mechanism or mechanisms and, since we are obliged to attempt an explanation for all of this, it is necessary to face this problem early. Are these effects of cortisone on so many different and unrelated hosts, infected by so many unrelated microorganisms, the result of one action of cortisone or of many different actions? This is a question of such central importance that it must be raised at the outset, even though it must quickly be added that we are not going to be able to answer it. It is actually the core of the matter. For, if we are dealing with a single general action of cortisone, this would imply that there exists in nature a single defense mechanism that is common to many species of host, and governs the resistance to infection by bacteria, viruses, protozoa, and fungi. The concept of such a mechanism is not new, but the availability of an experimental model with which to approach it is, and constitutes a major advance in the field of infectious disease. In this situation, generalizations from limited data are particularly hazardous, and it is a time to handle hypotheses with prudence and caution.

Before dealing with the problem of mechanism, there are several factors to be considered concerning the methods by which the effects of cortisone are demonstrated. The first of these is dosage. The amounts of cortisone required to bring about lethal infection are extremely large, in terms of what one expects for the biological effects of other hormones. The point has been made that we may be dealing with something like an artifact, an unnatural pharmacological effect that bears no real relation to any physiological function of the adrenal cortex. This is a fair point, but I am not sure of its meaning. The same thing could be said of the effects of cortisone on the symptoms of human diseases such as rheumatic fever or disseminated lupus, for the dosage employed is nearly comparable in terms of body weight. In Dr. Lurie's rabbits with tuberculosis the daily dose of cortisone was 2.5 mg. per kilo, and in our studies of Group A streptococcal infection,[4] a dose of 5 mg. was sufficient to cause lethal infection with regularity.

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* Some of the experimental studies described in this review were made in collaboration with Dr. Floyd W. Denny, Jr., Dr. Robert A. Good, Dr. Richard Smith, and Miss Joan Floyd, of the Department of Pediatrics, University of Minnesota Medical School. Support for these studies was provided by the United States Public Health Service, the Armed Forces Epidemiological Board, The American Heart Association, and the Minnesota Heart Association.

Dr. Robinson has raised the interesting possibility that smaller doses of cortisone might have a quite different effect on infection, perhaps causing protection. This would be of much importance if it could be established. It is very difficult at the present time to explain the increased susceptibility to infection of adrenalectomized animals on the basis of any deficiency involving cortisone or cortisonelike substances. If it could be shown that there is a dosage level at which cortisone has a beneficial effect, we would be greatly helped. Dr. Lurie has suggested that something of this sort may have happened in his experiments. With small doses of ACTH there was a suggestion of protection against tuberculosis in rabbits, in contrast with the disastrous effect of larger doses. A great deal more data are needed before the question can be settled.

TABLE 1

THE EFFECT OF ACTH ON INFECTION OF RABBITS BY GROUP A HEMOLYTIC STREPTOCOCCI

Group*	Number of rabbits	Number with septicemia	Number dead
ACTH†	8	7	7
No treatment	8	0	0

* All rabbits received an intradermal injection of 0.2 cc. of an 18-hour broth culture of streptococci.
† ACTH (Armour) injected in dose of 5 mg. every 6 hours, beginning one day before infection.

The question as to whether the effect of cortisone on infection represents an actual adrenal cortex function, or is a different pharmacological action, may be partly answered by the information obtained in experiments with ACTH. There are conflicts in the data which are probably due, as Dr. Finland suggests, to very large differences between the susceptibility of different animal species to the effects of ACTH. A striking example of this is the mouse, in which little or no change in resistance to pneumococcal infection can be demonstrated with extremely large doses of ACTH. But there is no question that ACTH has, in other species, the same effect as cortisone. Dr. Robinson has shown this in pneumococcal infections in rabbits; Dr. Lurie found it to be true for tuberculosis; and we encountered it in streptococcal infection.[4] In an experiment summarized in TABLE 1, ACTH was given in a dose of 5 mg. per kilo every 6 hours for one day before and after the intradermal injection of group A hemolytic streptococci. When injected into the skin of normal rabbits, this strain did not cause septicemia or death. In the ACTH treated animals, septicemia and death occurred in seven out of eight.

Several other hormones have been studied for their effects on infection, with results that speak well for the chemical specificity of the cortisone effect. Both Drs. Finland and Robinson have found that compound F causes enhancement of pneumococcal infection in mice and rabbits to the same degree as cortisone. Compound B was observed by Dr. Finland to produce a slight effect on pneumococcal infection, but none on influenza virus infection. Large doses of desoxycorticosterone combined with an augmented salt intake did not affect the resistance of rabbits to streptococcal infection in our laboratory.[4]

The action of growth hormone is a point of disagreement. Dr. Robinson has found, as was earlier reported by Selye,[17] that growth hormone antagonizes the effect of cortisone on infection, while Dr. Finland observed no such effect. In our laboratory, we were also unable to demonstrate any interference by growth hormone with the action of cortisone in streptococcus-infected rabbits. There is a difference between the basic nature of the experiments of Drs. Robinson and Selye, and those of Finland and myself, and perhaps the difference is important. They found that growth hormone prevented the *spontaneous* occurrence of pseudotuberculosis infections, which cortisone commonly activates from the latent state. In our experiments, in which growth hormone had no effect, we were inducing the infection after a period of cortisone pretreatment. Perhaps there is a difference between the activation of spontaneous infection and the enhancement of an induced infection, or between a pseudotuberculosis infection and one due to streptococci or pneumococci. The issue remains obscure, and more detailed bacteriological information is badly needed.

Another point that needs to be developed is the matter of the duration and timing of cortisone administration in relation to its action on infection. In early experiments on streptococcal infection in rabbits, it was noted that the most striking and reproducible results were obtained when animals were pretreated with cortisone for a period of three or more days before the infection. Using this technique, the majority of the treated animals developed septicemia and died and, when cortisone was not begun until the day of infection, the mortality was very much lower. It would be a mistake, however, to assume from these data that cortisone has no effect if given after infection instead of before. Recently, we found that the effect can be demonstrated when cortisone is given as late as 3½ months after infection.[18] This experiment is of sufficient interest to warrant an account of its details. Four rabbits were infected on June 5, by an intravenous injection of a type 24 strain of group A hemolytic streptococci. They survived without evident ill effects, and seemed to be entirely normal animals during the next month. At the end of July, almost two months later, daily blood cultures were made for one week, all of which were negative. At this time, cortisone was begun in a daily intramuscular dose of 25 mg. Four days later, two of the animals developed positive blood cultures for hemolytic streptococci. One strain was lost, but the other was identified serologically as group A, type 24. The bacteremia persisted for the next four days, at which time cortisone was stopped. After this, the blood cultures became negative again and remained so for the next month. In the middle of September, 3½ months after the original infection, cortisone was again started. Four days later, two of the four rabbits developed positive blood cultures for streptococci (one of these had had bacteremia in July, and the other not). The strain from one animal cannot be grouped or typed, but the other is group A, type 24. This experiment indicates that the effect of cortisone on infection is demonstrable for a considerable time after streptococcal infection. It also suggests a potential usefulness of cortisone in studying the unexpected chronicity and latency of infections by group A hemolytic strepococci.

Comparable results were obtained in infections by a nonhemolytic streptococcus, streptococcus MG, although here the time interval was shorter. A group of 12 rabbits were given an intravenous injection of a broth suspension of streptococcus MG. Two weeks later daily blood cultures were started, all of which were negative, and they were given cortisone each day. Within four days, three of the animals had positive blood cultures for streptococci and, in each instance, the organism was serologically identified as streptococcus MG.

Dr. LeMaistre has discussed the differences in the degree of alteration of the host–parasite relation produced in different animal species, and he suggests wisely that more than one animal be employed in studies of the cortisone effect. The point will, I am sure, find general agreement. Another matter worth raising is the difference between the effects of cortisone with unrelated varieties of bacteria. At first sight, it may seem that what cortisone does is to convert the experimental animal into a kind of test-tube culture. The distinguishing features of infections produced by different microorganisms are retained, however, despite the apparent failure of the host to respond. The course and outcome of the infection are no longer governed by the response of the host, but they are still influenced by the particular habits of the guest. The infections caused by different microorganisms resemble an exaggerated form of the kind of infection we could expect in each case. For example, the streptococcus is a relatively indolent pathogen for the normal rabbit and, once an infection is established, it lasts for a long time, while the pneumococcus kills rapidly. Under the influence of cortisone a streptococcal infection is still a protracted affair, and many animals survive for longer than a week, despite the presence of many thousands of organisms per cc. in colony counts of their blood, while the pneumococcal infection becomes more overwhelmingly and rapidly fatal. Similarly, brucella and typhoid infections are easily produced in rabbits with cortisone, but they remain the long-drawn chronic infections we expect with such organisms. With streptococcus MG we are unable to produce a lethal infection in normal rabbits, regardless of the numbers of bacteria injected and, although cortisone treatment brings about a transient bacteremia, it does not cause overwhelming infection or death. Perhaps, the situation is different with viruses, in view of the extremely rapid course of experimental poliomyelitis and Coxsackie virus infections that have been described during cortisone treatment. We are in need of much more data concerning the course of infection with many other species of virus.

Despite differences in the lethal effects of different infections, it is obvious that, in most cases, we are dealing with infections of enormous magnitude. In some instances, it is surprising that the host lasts for as long as he does. In cortisone-treated rabbits with hemolytic streptococcal infection, for example, although the animals seem surprisingly well for a week or more, and sometimes until almost the hour of death, daily colony counts of their blood show thousands of streptococci. They may continue to take their food and water and move about in the fashion of rabbits for day after day, with blood cultures showing hemolysis of the entire plate.

The local tissue reaction to bacteria seems to be markedly altered by cortisone, but not always in the same direction. Dr. Robinson found that the skin lesions produced by pneumococci were much larger in cortisone-treated rabbits. With skin infections by streptococci, cortisone prevents almost completely the usual inflammatory and necrotizing lesion that occurs in normal animals during the first two or three days. The observations of Turner[8] on the course of experimental rabbit syphilis during cortisone treatment are of interest. He found that, while the local lesions may appear for a time to be dampaned down, eventually they become very much more extensive.

We now arrive at the real task at hand, which is the consideration of mechanisms that may be involved in the action of cortisone on the process of infection. There are six possibilities that appear to merit individual attention. It is only fair to state in advance that there is not enough actual evidence to prove or disprove the case for any one of the six. There may be enough to permit the formation of openions as to the relative likelihood of each.

Possible Mechanisms

(1) The first possibility is that the effect of cortisone is exerted directly on the infectious agent itself, perhaps as a nutritional factor, for example, as or a stimulant of metabolic processes concerned with invasion or proliferation. On the face of it this seems improbable. It is difficult to imagine such an effect common to so many unrelated varieties of bacteria, not to say viruses, protozoa, and fungi. On the other hand, before we dismiss it altogether it would be prudent to remember that the hosts involved in the effect of cortisone also cover an extensive biological territory ranging from chick embryos to man. Actually, the only thing that can safely be said about the role of the agent is that to date no effect of cortisone on any microorganism has been domonstrated that might be expected to alter the capacity to infect. The possibility has not been excluded, but we are certainly at liberty to consider other mechanisms involving the host.

(2) The second possibility is that, in some way, the polymorphonuclear leukocyte is altered by cortisone. It is known that animals deprived of granulocytes by such poisons as benzene or nitrogen mustard become extremely susceptible to lethal bacterial infections. It is also known that these cells are capable of destroying bacteria in the circulating blood, and there is abundant evidence for their bactericidal action *in vitro*. Moreover, in the tissue infections of cortisone-treated animals, such as the pneumococcal skin infection of rabbits described by Dr. Robinson, it is apparent that granulocytes do not accumulate in infected areas as rapidly or in such numbers as in normal animals. These may be taken as points in favor of an effect of cortisone on the granulocyte. On the other hand there are several good reasons for doubt. For one thing, no one has yet demonstrated that the phagocytic or bactericidal properties of polymorphonuclear leukocytes are in any way affected by cortisone. Moreover, the numbers of these cells in the blood are not reduced by cortisone. In fact, they are usually increased. The antibacterial function of

granulocytes in local infections is generally accepted, but there is some question as to the importance of their role in clearing the blood of bacteria. Kerby and Martin[19] for example, have recently shown that the rate of removal of staphylococci from the blood in rabbits is not significantly depressed when extreme polymorphonuclear leukopenia is produced by benzene or nitrogen mustard. These workers suggest that granulocytes may have their phagocytic activity at the local source of infection in tissues, while some other mechanism is concerned with blood stream infections. Finally, apart from the question of granulocytes as antibacterial agents, there is little evidence that these cells are of importance in defense against viruses, protozoans, or fungi. In short, there is not yet enough data to implicate this group of cells, and not enough to rule it out.

(3) The third possibility is that the effect of cortisone is mediated through its action on the antibody-forming mechanism. It has been shown in a number of different immune systems that cortisone-treated animals fail to produce antibody as quickly or in as high titers as normal animals. Also, the level of an already existing antibody has been shown to be significantly depressed during cortisone treatment.[20, 21] On the basis of these observations, it might be suggested (and it has been) that the enhancement of infection is the result of interference with the formation or maintenance of adequate antibody. There is one great difficulty here, and it concerns the time factors involved. In most of the infections that are enhanced by cortisone, events move very rapidly after the introduction of microorganisms. For example, when cortisone-treated rabbits are infected in the skin with group A streptococci, an extensive septicemia becomes established within less than 24 hours. In normal animals there may be a transient bacteremia a few hours after infection but, by the end of 24 hours, the blood cultures are negative. It is difficult to imagine that the prevention of septicemia in the normal rabbit is a function of antibody formation, unless one assumes that a brand-new antibody can be stimulated, make its appearance and block a formidable infection, all within less than a day. The situation is similar in other types of infection, such as those produced by pneumococci, staphylococci, and the poliomyelitis and Coxsackie viruses. The enhancement of infection occurs too rapidly and is established too early to implicate any conventional antibody mechanism.

Another reason for doubt is that cortisone treatment has been found to cause little or no enhancement of infection in animals previously immunized against the microorganism employed, as has been reported by Dr. Finland. Similar observations were made by us in experiments with streptococcal infection.[4]

The enhancement of virus infection produced by cortisone in chick embryos is an additional piece of evidence against the implication of antibody, since it is generally believed that the embryo does not possess an antibody-forming mechanism during this period of development.

(4) The fourth possibility is that interference by cortisone with the inflammatory reaction in tissues is the basis for its influence on infection. The depression of tissue inflammation by cortisone is an accepted fact, although the mechanism by which it occurs remains unexplained. It is probable that

inflammation represents an effective mechanism for resistance against infection in tissues and for the limitation of infection. A depression of the local inflammatory response in cortisone-treated animals might account for the spread of organisms from infected tissues, such as the skin, to the general circulation. But it is not clear how this would explain the enhancement of infections in which there is no recognizable stage of local inflammation, such as virus infections, or bacterial infections produced by the injection of organisms directly into the blood. Also, it is difficult to see how an impairment of inflammation can account for the activation of latent infections in apparently healthy animals. It is of course possible that a cellular component that is involved in the inflammatory cycle may also operate against viruses or bacteria in the blood stream, and cortisone may affect both activities of such a cell. The question must remain open until this kind of information becomes available.

In connection with the inflammatory reaction, the possible role of changes in the permeability of small blood vessels has also been suggested to explain the effect of cortisone. Dr. Lurie has raised the point that a reduction of capillary permeability may be caused by cortisone, and data to support this view have been obtained in other quarters.[22] Such a change might greatly alter the picture in a tissue inflammatory reaction, but it does not readily explain the events in systemic bacterial or virus infections. As in the case of inflammation itself, the subject is not closed, and neither does it appear to be invitingly open, at the moment.

(5) A fifth possibility is that other serological mechanisms concerned with defense against infection may be impeded by cortisone. We have in mind a group of poorly understood phenomena, including complement, the so-called "natural" bactericidal antibodies, and a congeries of substances that appear in blood during the acute phase of infections,[23] such as C-reactive protein, Tillett's bactericidal factor, hyaluronidase inhibitor, and serum mucoprotein. Some of these are known to be affected by cortisone. Hyaluronidase inhibitor, for example, rises during cortisone treatment in normal animals,[24] and the level of C-reactive protein in human beings with rheumatic fever is promptly reduced by cortisone.[25] We know too little about the meaning of these substances. In fact, all we can say is that they exist and that, if they do have a function in infection, it has not yet been identified.

(6) Finally, the sixth mechanism to be considered is the reticuloendothelial cell system. This complex apparatus is distributed throughout many organs in the body, and is composed of phagocytic cells derived from the mesenchyme. It includes the fixed macrophages in the endothelium of the spleen, liver, lymph nodes, and bone marrow, and the resting or motile macrophages in all tissues. These cells were classed as a single system by Aschoff, because of their common property of taking up and removing from the blood stream large quantities of vital dyes, colloidal and other particulate materials, and bacteria. The system appears to be the principal and perhaps the only mechanism for the clearance of such things from the blood. For this reason, the reticuloendothelial cells have long been regarded as a major defense mechanism against infection in general. It has been demonstrated repeatedly that bacteria in-

jected into the circulation are rapidly removed, and they can be seen within these cells within a few hours after injection. Enders,[26] for example, showed that injected pneumococci disappeared within one or two hours from the blood stream and, during this period, were engulfed and killed by the RE cells. Similar observations have been made with pneumococci, staphylococci, streptococci, and paratyphoid bacilli by Cannon,[27] Gay,[28] Keyes,[29] Wright,[30] and others. Recently, Wood and his co-workers[31] have found reason to doubt the relative importance of this clearing mechanism and have emphasized the role of polymorphonuclear leukocytes in the early removal of pneumococci. Contrary data mentioned previously were reported by Kerby and Martin[19] from experiments with leucopenic rabbits injected with staphylococci, in which the absence of granulocytes did not affect the clearance rate.

Dr. Robinson has indicated that cortisone treatment interfered with the early clearance of pneumococci from the blood in rabbits, and suggests involvement of the RE system. Dr. Lurie has also suggested that these cells may be defective, but on somewhat different grounds. His interpretation of the events in tuberculous rabbits in that the phagocytic activity of the RE cells is actually increased, but that the capacity of the cells to digest tubercle bacilli is lacking. This view is based, in part, on the increased degree of phagocytosis observed by Dr. Lurie in the lungs of tuberculous cortisone-treated rabbits as well as an increase in the phagocytosis of carbon particles by glomerular capillary cells during cortisone treatment. Recently, Germuth[32] has presented evidence indicating that the cortisone-treated rabbit exhibits no impairment of the mechanism for clearance of pneumococci from the circulating blood. It should be pointed out, however, that the removal of substances from the blood may not constitute the only important manifestation of the functioning of the reticuloendothelial system. Of greater importance, may be the tissue areas in which, under various influences, the substances are taken up.

There are several rather general reasons for suspecting that the normal operation of the RE system may be interfered with by cortisone. On morphological grounds, the lymphoid tissues, which contain the bulk of this system, seem to be a major target of cortisone. Lymphoid organs, such as the spleen and lymph nodes, shrink rapidly in size during treatment, and histological evidence of dissolution of the cells in these tissues has been demonstrated.[33, 34] What this means in terms of the number or function of RE cells remains to be determined, but it could mean much. The number of lymphocytes in the blood is greatly reduced, sometimes to zero. If these cells constitute the source of tissue macrophages, as many believe, this would create a major functional defect in the RE system. Moreover, the cells of this system are probably involved, directly or indirectly, in the antibody-forming mechanism, which is profoundly altered by cortisone. It is also a source of important cellular participants in the inflammatory cycle, and it may provide parent cells for the fibroblasts which comprise the basic reaction in wound healing. On these grounds, several unrelated manifestations of cortisone might be accounted for by an effect on one cell system. Also, the RE system is sufficiently universal in its distribution in nature to account for the wide range of hosts that are affected by cortisone.

A major difficulty in all studies of the RE system has been the absence of direct experimental methods for the quantitative evaluation of phagocytosis *in vivo.* An indirect approach employed by many workers has involved the production of so-called RE "blockade," by the injection of colloidal materials such as thorotrast, trypan blue, India ink, or iron saccharate. These substances are rapidly removed from the blood and simultaneously become concentrated with RE cells, chiefly in the liver, spleen, and lymph nodes. It has been postulated that as the result of one such injection, the capacity of the cells to take up another substance becomes temporarily impaired or lost—hence the term blockade. The literature, especially that of several decades ago, is filled with conflicting reports concerning this phenomenon. It has been claimed, and denied, that many varieties of bacterial infection are enhanced by RE blockade. Recently, Martin[35] has shown that the clearance of staphylococci from the splanchnic circulation of rabbits is delayed when thorotrast is injected prior to the injection of bacteria.

One of the materials that appear to be entirely removed from the blood by RE cells is colloidal gold, and this has provided a quantitative method for evaluating the function of these cells.[36] By the use of radioactive gold as a tracer, it is possible to obtain reproducible curves of the rate of removal in normal rabbits and in animals subjected to RE blockade.

In the text to follow, we shall speak of "blockade" of the RE system, and of "blockading agents." It should be made clear at the outset that these terms are used as conveniences, but they are not to be interpreted as meaning that the RE system as a whole is inhibited or prevented from taking things out of the circulating blood. The recent studies of Halpern[37] have emphasized the possibility that a shifting of certain general functions of the system from one organ to another may be of greater importance than any change in the rate of total blood clearance. This worker has shown that the rate of removal of India ink, for example, is not significantly reduced when progressively larger doses of ink are injected, but that the particular organ in which the material is mainly removed changes with changing doses. With small doses, the liver removes the majority. As the dose is increased, the rate of disappearance from the blood remains the same, but the spleen, lungs, and kidneys enter the picture as clearing mechanisms. Thus, instead of considering "blockade" by an injection of India ink to be a matter of prolonged retention of subsequently injected material in the blood, it might be regarded as a mechanism for the shifting of phagocytic functions to endothelial cells in tissues not normally concerned with such activities. An extension of this concept to the problem of cortisone would permit an explanation for certain observations that seem irreconciliable otherwise. For example, some of the data to be presented below suggest that the effects of cortisone resemble those of certain agents causing RE blockages. On the other hand, there is Dr. Lurie's observation of greatly enhanced uptake of India ink by glomerular capillary cells. Perhaps, this observation does not mean that the over-all functioning of the RE system is stimulated by cortisone, but that part of the system has been interfered with and that new tissues with phagocytic capabilities have become active as the result. Perhaps, in order to fit this concept into the problem of cortisone and infection, the efficiency of phagocytosis as a bactericidal or bacteriostatic device may be high in some tissue cells and low in others.

After this preamble, we shall consider a set of rather startling similarities between the reactions of cortisone-treated rabbits and animals that have been subjected to RE blockage. The resemblances, which seem more than coincidental, are demonstrated in three different unrelated experimental situations. The first similarity is in the effect on bacterial infection; the second is in the effect on the rate of clearance of radioactive colloidal gold from the blood; and the third is in the effect on the response to gram-negative bacterial endotoxins.

The facts concerning cortisone and streptococcal infection[4] may be briefly recapitulated as follows. Normal rabbits are resistant to an intradermal streptococcal infection and, in most instances, septicemia and death do not occur. Rabbits treated with cortisone for three days before infection are highly susceptible, and septicemia and death occur in the majority.

The skin lesions are strikingly different in the two groups. During the first two or three days after infection, the cortisone-treated animals show little or no reaction at the injected area. During the same period, the control animals develop larger and much more extensive local reactions, with redness, swelling, and necrosis.

The effect of thorotrast[38] on streptococcal skin infection was studied in the following manner. Thorotrast* was given by vein, in a dose of three cc. per kilo and, several hours later, an intradermal injection of streptococci was made. At the same time, untreated animals were similarly infected. The dosage of streptococci was sufficient to cause extensive inflammatory and necrotic lesions in all the controls. In the thorotrast-treated rabbits, the reactions at the skin site were entirely different, and closely resembled those seen in cortisone-treated animals. There was little or no edema, no erythema, and only occasionally did small areas of necrosis occur in the center of the area.

The effect of a systemic streptococcal infection was greatly enhanced by thorotrast. TABLE 2 illustrates an experiment in which 10 rabbits were injected with thorotrast and then given varying dilutions of a streptococcal broth culture by vein. At the same time, 10 untreated rabbits were infected in the same manner. Within 48 hours, all of the thorotrast group were dead, with heavily positive blood cultures. None of the control animals died.

Similar observations with pneumococcal infections were made in our laboratory by Dr. Robert Good. A relatively avirulent strain of type 1 pneumococcus was injected intradermally in rabbits that had received, several hours earlier, an intravenous injection of thorotrast or a colloidal solution of saccharated iron oxide.† At the same time, control animals were similarly infected. The results are shown in TABLE 3. In the control group, 26 of 27 developed a conspicuous local skin reaction, with redness, edema, and some hemorrhage; only two of these animals died within the next 48 hours. In 18 rabbits given thorotrast before infection, only one showed a local skin reaction and all were dead within less than two days. In three rabbits that received intravenous iron saccharate, in a dose of 1 cc. per kilo, followed by pneumococci, there were no skin reactions and all died.

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* Thorotrast is a colloidal solution of thorium dioxide, containing 24–26 per cent thorium dioxide, manufactured by Heyden Chemical Corporation, New York. Lot numbers 202 through 211 were used in these experiments.

† The preparation of saccharated iron oxide was a solution containing the equivalent of 20 mg. of available iron, designated Proferrin, manufactured by Sharp and Dohme, Philadelphia, Pennsylvania. The material was injected by vein in a dose of 1 cc. per kilo.

These results would suggest that RE blockade by thorotrast or iron saccharate causes enhancement of infection, and could be taken as a point of analogy between the action of such materials and cortisone, provided that we were certain that the blockading agents do not do a great many other things to other defense mechanisms, and that they really do produce blockade. Regrettably, we do not really know either of these things. It is entirely possible that thorotrast or iron saccharate may have an effect on granulocytes (although no decrease in the white-cell count occurred), or on complement or some other unknown mechanism. As to the question of blockade, we are on firmer ground.

TABLE 2

THE EFFECT OF INTRAVENOUS THOROTRAST ON INFECTION BY GROUP A STREPTOCOCCI IN RABBITS

Dose of streptococci[*]	Lethal septicemia	
	Thorotrast treated[†]	Untreated
10^{-2} i.v.	3/4	0/3
10^{-4} i.v.	4/4	0/4
10^{-0} i.d.	3/3	0/3

[*] Dilutions made from 18 hour broth culture of streptococci. i.v. = intravenous injection of 1 cc.; i.d. = intradermal injection of 0.2 cc.

[†] Thorotrast (Heyden Chem. Co., lot no. 211) injected in dose of 3 cc. per kilo, 4 hours prior to the injection of streptococci.

TABLE 3

THE EFFECT OF THOROTRAST AND COLLOIDAL IRON SACCHARATE ON PNEUMOCOCCAL INFECTION IN RABBITS

Group[*]	Number of rabbits	Skin reaction[†]	Death[‡]
Thorotrast..................	18	1	18
Iron saccharate...........	3	0	3
No treatment.............	27	26	2

[*] Thorotrast, 3 cc. per kilo, and Iron Saccharate (Proferrin, Sharp and Dohme,Inc.) 1 cc. per kilo, injected intravenously 4 hours before infection. All rabbits received an intradermal injection of a suspension of type 1 pneumococci of low virulence.

[†] Refers to the local inflammatory reaction at the site of injection of pneumococci.

[‡] Numbers of rabbits dying within 36 hours after the injection of pneumococci.

It has been shown by Barrow, Tullis, and Chambers[39] with radioactive gold tracer that the intravenous injection of thorotrast causes a marked delay in the clearance from the blood of colloidal gold, a substance that is apparently removed almost entirely by the liver and spleen. Confirmatory observations have been made in our laboratory by Dr. Samuel Cornwall. Moreover, cortisone produced a similar delay in the clearance of colloidal gold from the blood. In Dr. Cornwall's experiments, the rate of uptake of gold was determined by injecting colloidal gold with a radioactive tracer intravenously, and taking repeated samples of blood at frequent intervals thereafter. It was found that both cortisone and thorotrast caused a comparable degree of delay in the removal or uptake of colloidal gold from the circulating blood.[40]

Finally, a third and quite different experimental situation involves the reaction of rabbits to gram-negative bacterial toxins, and the alteration of this reaction produced by cortisone. The experiments to be described have been presented in detail in other publications.[41-43]

When a rabbit is injected intradermally with a preparation of meningococcal toxin similar to that employed in the Shwartzman reaction, the local skin response is one of mild induration and some erythema. In rabbits treated for three or more days with cortisone, in a dosage of 25 mg. daily, this mild reaction does not occur. Instead, numerous small petechial hemorrhages appear at the injected skin site on the following day. As we will see in a moment, lesions involving internal organs also occur in such animals.

When the toxin is injected by vein in normal rabbits, no visible changes are produced in the internal organs. But when a single intravenous injection of toxin is given in cortisone-treated rabbits, the animals develop bilateral cortical necrosis of the kidneys,[41] as well as hemorrhages in the lungs, liver, spleen, and gastrointestinal tract. The basis for cortical necrosis appears to be the accumulation, within the glomerular capillaries, of homogeneous pink material with the staining properties of fibrinoid. This material is deposited in the glomeruli before there is any evidence of necrosis elsewhere in the kidney, and it is presumed to occlude the blood supply to the cortical tubules.

This kidney lesion is the same as that of the so-called generalized Shwartzman reaction, except that the latter is only produced when *two* intravenous injections of toxin are given 24 hours apart. It does not occur after a single injection, regardless of the amount of toxin employed. In addition to the morphological similarity, the cortisone-induced kidney lesion and the generalized Shwartzman reaction also resemble each other in that both are completely prevented by the administration of nitrogen mustard,[41] and by heparin.[44]

The hemorrhagic skin reaction produced by toxin in cortisone-treated rabbits is quantitatively milder than the true local Shwartzman reaction, but histologically there is a resemblance between the two. In the lesion of the cortisone-treated rabbits, the small veins in the involved skin are occluded by dense aggregates of leukocytes and platelets, a lesion shown by Stetson[45] to be characteristic of the local Shwartzman reaction.

In addition to these effects, there is another action of cortisone on the response to gram-negative bacterial toxin that seems of much importance. When meningococcal toxin is injected into the skin of normal rabbits, there is evidence that little if any is absorbed into the general circulation. The animals do not become ill, regardless of the amount of toxin injected, the Shwartzman reaction cannot be provoked when the second injection is given into the skin instead of by vein, and no lesions occur in internal organs after an intradermal injection. Apparently, the skin possesses an efficient mechanism for the detoxification or fixation of toxin. This is not the case in cortisone-treated rabbits, and the capacity to retain toxin in the skin is lost. This observation is illustrated in TABLE 4. Four groups of rabbits are shown in the table. The first group received two intravenous injections of 0.4 cc. of *S. marcescens* toxin, and all four animals developed the generalized Shwartzman reaction. In the second group, four rabbits were prepared for the generalized Shwartzman reaction by the same dose of intravenous toxin, but the second injection was given intradermally instead of by vein. None of the animals became ill or developed kidney lesions. This indicates that the toxin placed in the skin was not absorbed in an amount sufficient to provoke the reaction. The third group indicates the same sort of thing, but here both injections of toxin were made in the skin. In the fourth group, the effect of cortisone is illustrated. Here, eight rabbits received cortisone for three days and were then given a single intradermal injection of the same dose of toxin. All of these animals exhibited hemorrhages at the injected skin site, and seven of the eight developed bilateral cortical necrosis of the kidneys. We may infer from this experiment that cortisone not only makes it possible for a single injection of toxin to cause renal necrosis, but that it also permits the absorption of toxin from the skin, interfering with a mechanism that, in the normal rabbit, is responsible for the retention or detoxification of toxin in the skin.

TABLE 4

THE PRODUCTION OF BILATERAL CORTICAL NECROSIS OF THE KIDNEYS BY INTRADERMAL *S. marcescens* TOXIN IN CORTISONE-TREATED RABBITS

Group	Procedure	Number of rabbits	Number with renal cortical necrosis
I	Two intravenous injections of 0.4 mg. *S. marcescens* toxin	4	4
II	Intravenous toxin followed by intradermal toxin	4	0
III	Two intradermal injections of toxin	4	0
IV	One intradermal injection of toxin in cortisone-treated rabbits	8	7

This, then, is the basic experimental model with cortisone. The cortisone-treated rabbit develops skin hemorrhages after an intradermal injection of meningococcal toxin and this lesion resembles the local Shwartzman reaction. The cortisone-treated rabbit develops renal cortical necrosis, identical to that of the generalized Shwartzman reaction, after a single intravenous injection of toxin. Finally, cortisone permits the absorption of toxin from the skin into the blood stream, an event that does not occur in normal rabbits.

Having shown that the enhancing effect of cortisone on bacterial infection may be imitated by substances that are taken up by the RE cells, and that both thorotrast and cortisone cause a delay in the clearance of colloidal gold from the blood, it was next of importance to determine whether the phenomena produced by gram-negative bacterial toxins in cortisone-treated rabbits can be imitated in animals subjected to RE "blockade."

First, the effect of thorotrast on the skin reaction to intradermally injected toxin.[43] TABLE 5 shows what happened to six rabbits that received thorotrast intravenously, followed six hours later by an intradermal injection of meningococcal toxin. It will be seen that four of the six died; all six developed skin hemorrhages similar to typical severe Shwartzman reactions; and all six developed bilateral cortical necrosis of the kidneys. There is another point of resemblance between these reactions and the skin and kidney lesions caused by toxin in cortisone-treated rabbits. Nitrogen mustard, in a dose sufficient to produce granulocytopenia, protected the thorotrast-treated animals against the lethal effect of gram-negative toxin, as well as against the skin lesions and renal necrosis.[43]

TABLE 5

THE PRODUCTION OF CUTANEOUS HEMORRHAGE AND BILATERAL CORTICAL NECROSIS OF THE KIDNEYS BY A SINGLE INTRADERMAL INJECTION OF MENINGOCOCCAL TOXIN IN THOROTRAST-TREATED RABBITS

Group	Number of rabbits	Number with cutaneous hemorrhage	Number with renal cortical necrosis
Thorotrast-treated*...........	6	6	6
Untreated....................	6	0	0

* Thorotrast given in dose of 3 cc. per kilo, 6 hours before the intradermal injection of meningococcal toxin.

TABLE 6

THE EFFECT OF THOROTRAST ON THE LETHAL ACTION OF INTRAVENOUSLY INJECTED MENINGOCOCCAL TOXIN

Group	Dilution of toxin	Number of rabbits	Number dead	Number with renal cortical necrosis
Thorotrast treated	1–20	10	10	0
	1–40	6	6	0
	1–160	9	9	3
	1–640	6	6	3
	1–2560	6	4	5
	1–5120	6	3	4
Untreated	1–20	10	1	0
	1–40	6	0	0
	1–160	4	0	0
	1–640	4	0	0
	1–2560	4	0	0
	1–5120	4	0	0

Quantitatively, the thorotrast effect was greater than that of cortisone in two respects. First, the skin and kidney lesions were more intense than those seen with cortisone. Second, the amounts of toxin required to cause renal necrosis, and death were very much smaller than those effective in cortisone-treated rabbits. This is illustrated in TABLE 6, which shows the effect of various dilutions of meningococcal toxin, given as a single intravenous injection, in normal and thorotrast-treated rabbits. One rabbit in the untreated series died after an injection of toxin in a dilution of 1–20. No renal lesions occurred in any of the animals. In the thorotrast-treated group, death occurred in the majority of animals following injections of toxin in dilutions ranging between 1–20 and 1–5120. Bilateral cortical necrosis of the kidneys was produced by the dilution of 1–5120 in four of six animals. Negative kidneys were encountered in rabbits receiving the largest concentrations of toxin, due to death of these animals shortly after the injection of toxin and before the kidney lesion had time to develop.

In all of these experiments, it was essential that thorotrast be given first, and then toxin. When the order was turned around, and toxin given before thorotrast, no deaths or lesions in the skin or kidneys occurred with any amount of toxin. The optimal time for injecting toxin was about six hours after thorotrast, but the effect was still demonstrable as late as 48 hours after thorotrast.

Three other colloidal substances, trypan blue,[43] India ink,[46] and iron saccharate,[46] produced similar results. These were not as active as thorotrast, and somewhat larger doses of toxin were required, but the basic effects were the same. Following an injection of either trypan blue, colloidal carbon, or iron saccharate, the lethal effect of a single intravenous injection of toxin was markedly enhanced, and bilateral cortical necrosis of the kidneys occurred in some of the animals. Similarly, an intradermal injection caused hemorrhagic necrosis in the skin of animals previously injected with these materials.

In summary, the observations indicate that the effects of cortisone on the response to bacterial infection and on the action of gram-negative bacterial toxins may be imitated, during a crucial period of time, by colloidal materials capable of "blockading" the reticuloendothelial system.

The following hypothesis would be consistent with the data: The effect of cortisone is to interfere with the functioning of the RE system, impairing its normal capacity to remove, fix, or detoxify bacteria and certain bacterial products. The result may be a shifting of these functions to tissues not normally concerned with them, with uncontrolled infection or tissue damage as the outcome.

As a possible mechanism, this concept is in the same position as the other five discussed in this review. There is not yet enough direct evidence to prove or disprove it. As is also the case with the others, it is useful as a working hypothesis, and it has raised interesting questions, which seem to be experimentally approachable.

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